BIOAVAILABILITY STUDIES ON ORALLY ADMINISTERED, OILY SUSPENSIONS OF DRUGS

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MY PARENTS

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Author's Declaration

During the course of the research programme I have not been registered for any other award of the CNAA or of a University. Furthermore, none of the material contained in this thesis has been used in any other submission for an academic award.

ABSTRACT

of a Ph.D. thesis (CNAA) entitled

"Bioavailability studies on orally administered,oily suspensions of drugs".

by

Omran M.O.Alhammami

Bioavailability studies on suspensions of sodium salicylate, nitrofurantoin and ampicillin in various oily vehicles,based on Fractionated Coconut Oil (FCO),have been carried out in either rabbits or rats. In vitro dissolution rate tests have also been performed, together with rheological, solubility, adsorption and partition coefficient measurements.

The results suggest that the viscosity of the vehicle plays an insignificant role and that most of the observed changes occurring in vivo can be attributed to the delaying effects of FCO on the gastric emptying rate (GER). The results of this delay depend on the pK_a value and solubility of the drug. In the case of ampicillin, stimulation of a biliary recycling process by the oil also appears to affect the plasma concentration versus time curve.

The inclusion of sucrose in FCO also leads to significant changes when the apparent partition coefficient of the drug between FCO and 0.1 mole/dm^3 HCl is high, as it is for sodium salicylate, but not when it is low, as with ampicillin. It is suggested that the bioavailability increasing mechanism of sucrose is caused by the effect of high osmotic pressure on the uptake of water by the GI membrane and is not due to an additional delay in GER over that caused by the oil itself. The enhancing effect of sucrose on the bioavailability of salicylate is nullified by the inclusion of 1% Cab-o-sil and it is suggested that adsorption of the drug on to Cab-o-sil is responsible for this effect.

The in vivo bioavailability parameters correlated poorly with in vitro parameters. It is suggested that such correlation indicates that traditional dissolution rate tests, such as dialysis and flask-stirrer methods, are unsatisfactory as bioavailability indicators when applied to dosage forms that cause marked changes in physiological factors like GER and biliary excretion.

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SECTION I

INTRODUCTION

CHAPTER I

BIOAVAILABILITY

1.1. Definition and significance of bioavailability

The term bioavailability is usually defined as the rate and extent of absorption of a drug from its dosage form into the systemic circulation (Blanchard and Sawchuk, 1979). In the context of this definition, general circulation refers primarily to venous blood (excepting the hepatic blood during the absorption phase) and arterial blood which carries the drug to the tissue (Gibaldi and Perrier, 1975).

According to the above definition, the bioavailability of an intravenously administered drug is rapid and complete. However, for reasons of convenience and stability, most drugs are administered orally after first being formulated into a dosage form (delivery system), which is usually a tablet or a capsule. In these circumstances the rates and extents of absorption of the drugs in an individual are usually not precisely known for reasons that are given later in this section.

Other terms that have identical or similar meanings to 'bioavailability' have also been referred to in the literature, e.g. 'physiological availability' (Oser et al, 1945), 'efficacy of absorption' (Wagner, 1971a), 'biophasic availability' (Smolen, 1971), 'systemic availability' (Barr, 1973) and the more general term 'biological availability'.

Since it is generally assumed that the therapeutic effect of a drug is a function of the concentration of the drug in a patient's blood (or plasma or serum) the importance of bioavailability in drug

therapy stems from the fact that the rate and extent of absorption of an administered drug can, in principle, affect the patient's response to the drug.

The intensity of pharmacological response elicited by many drugs is probably directly related to the concentration or activity of the drug in the immediate vicinity of the receptor site in the blood (i.e. biophase) (Ariens, 1964). The term biophasic availability has been used by Smolen (1971) to describe the availability of a drug in its biophase. Unfortunately, this approach is only useful for drugs whose intensity of action can be easily and accurately determined, and for the majority of drugs this approach tends to be difficult and of limited sensitivity. Furthermore, it is often not possible to measure the drug concentration directly by sampling from the biophase, therefore the concept of biophasic availability is presently more useful than its application, at least for the majority of the drugs (Koch-Weser, 1974).

The clinical significance of bioavailability depends on the assumption that an apparent distribution equilibrium is established between drug in the blood and receptor compartments. Once this equilibrium has been attained, measurement of the concentration of drug in the blood is assumed to provide an indirect measure of the concentration of drug at the receptor site (Koch-Weser, 1972; Chasseaud and Taylor, 1974). Alternatively, urinary excretion of the unchanged drug can be measured (Koch-Weser, 1974; Ritschel, 1980c;Gibaldi, 1977a; Sjoqvist et al, 1980). Hence, the determination of the blood concentration or urinary excretion data of the drug may allow one to follow the time course of pharmacological activity. However, this is

not always the case, since there is no guarantee that a drug reaching the systemic circulation will also reach the receptors in adequate concentration. It is possible to identify some instances in which systemic availability may not necessarily be the same as pharmacologic or biophasic availability. This will occur when the biophase is in a poorly accessible region, such as a deep-seated infection in a poorly perfused tissue, which does not rapidly reach distribution equilibrium with the blood compartment. An example of a situation where direct measurements on the biophase do show poor initial equilibration is seen in the work of Sholkoff et al (1967) on salicylate concentration in synovial fluid, since these do not parallel plasma concentration.

In spite of this limitation to the clinical significance of systemic availability use is often made of bioavailability measurements in the assessment of pharmaceutical products. A knowledge of the factors that influence bioavailability is therefore important. Barr (1973) reported that the amount of drug reaching the systemic circulation is a function of, but not necessarily equal to, the amount of drug available for absorption from the gastrointestinal (GI) tract. This function is dependent on a number of factors that may consequently affect the bioavailability. These can be conveniently classified as either patient related or dosage-form-related factors (Barr, 1973; Koch-Wesser, 1974; Wagner, 1977; Blanchard and Sawchuk, 1979) or, alternatively, following Ritschel's nomenclature (1980c), as factors causing either physiologically modified bioavailability or dosage form modified bioavailability, respectively. The former factors include, for example, the effects of stomach emptying rate, intestinal transit time, bile salts, mucin, blood flow to the GI tract, variation in the pH of the

GI fluids, intestinal metabolism and recycling processes. The dosage-form-related factors include formulation and manufacturing variables, in other words physicochemical properties of the dosage forms, such as particle size, the chemical form and solubility of the drug and the type of the vehicle or excipient that is used.

Knowledge of these factors is essential for proper interpretation and evaluation of bioavailability studies since a difference found in bioavailability between two different drug products may be falsely attributed to dosage form factors when a physiologically modified bioavailability is the correct cause, unless the change in the formulation causes an alteration in physiological function.

The possible effects of dosage-form-related factors on the bioavailability of drugs has led to the concept of the bioequivalence of drug products. Thus, if two products containing the same amount of the same therapeutically active ingredients in the same dosage forms (i.e. chemically equivalent or pharmaceutically equivalent) produce different bioavailabilities they are said to be bioinequivalent. If the bioavailabilities are similar then the products may be described as being bioequivalent. If the comparison is made of the therapeutic and/or toxicity effects then it is usual to talk in term of therapeutic equivalence or inequivalence rather than bioequivalence (Blanchard and Sawchuk, 1979).

1.2 Methods of assessing bioavailability

1.2.1 In vivo methods

The pharmacological responses elicited by some drugs may be used to provide an assessment of bioavailability (Koch-Weser, 1974;

Wagner, 1975c);e.g. the lowering of blood sugar by antidiabetic agents and the lowering of blood pressure by hypotensive agents. However, this approach cannot be used for many drugs and, consequently, most bioavailability assessments are based on the determination of the concentration of the drug, and/or its metabolites, in samples of blood and/or urine (Gibaldi and Groundhoffer, 1975; Wagner, 1977; Dittert and DiSanto, 1978; Sjoqvist et al, 1980), that are taken at specified times after administration of the drug or drug product to the subject, which may be a laboratory animal, a human volunteer or a patient. Plots of the resultant data yield curves describing the time course of the drug in the body fluid and are often referred to a blood level curves, urinary excretion curves, etc.

Comparisons of parameters derived from blood level curves with those derived from a similar curve obtained after administration of a reference formulation allow an assessment of the bioavailability of the drug in the test formulation to be made. If the reference formulation is an intravenous injection then it is accepted that the method yields an absolute assessment but if an extravasally administered reference formulation is used, e.g. an orally administered solution or standard formulation of proven clinical efficacy, then the bioavailability of the drug in the test formulation is generally assessed in a relative manner. However, it is possible to obtain an estimate of the absolute bioavailability of certain drugs without using an intravenous injection as the reference formulation by using Lalka and Feldman's method (1974). This method is based on the concept of renal clearance and involves perturbation of the clearance of a drug by the co-administration of urinary acidifying or alkalinizing agents. A modification of this method has

been proposed by Barzegar-Jalali (1980), which avoids the need for frequent blood sampling.

The parameters that are usually derived from blood level curves and used in bioavailability assessments are (i) the area under the curve (AUC), (ii) the peak time (PT) and (iii) the peak concentration (PC). A comparison of AUCs for test and reference formulations yields an estimate of the extent of absorption, whereas an analysis of the peak times and peak concentrations provides an evaluation of the rate of absorption (Gibaldi, 1977a, Kaplan and Jack, 1979).

The information obtained from the blood level curves of a drug may often be supplemented by estimates of drug excreted in the urine. In fact, if an assay of the drug in blood is either unavailable or unreliable urinary excretion data may provide the only effective measurement of bioavailability, e.g. for nitrofurantoin (Cadwallader et al, 1978). It is usually recommended that the amounts of intact drug and its metabolites in the urine should be determined.

However, Ritschel (1980b) has shown that if at least 10% of the drug is excreted intact then measurement of this intact drug alone is adequate for estimation of the extent of bioavailability.

Although an indication of the rate of bioavailability can be deduced from urinary excretion curves the practical data is often not very precise because of the difficulties that arise in the collection of samples at given times and frequencies. These ,difficulties can be overcome by catheterisation of the subject.

Since the results obtained in an <u>in vivo</u> bioavailability assessment will be influenced markedly by the biological variability between subjects, then an adequate experimental design is essential

in order to minimise this influence and allow its effect to be taken into account in the analysis of the results. The design of comparative bioavailability tests and analysis of their results has been reviewed by Westlake (1973 and 1979).

1.2.2 In vitro methods

The in vivo methods of assessment that are mentioned in the previous section are expensive and time-consuming. Consequently, many attempts have been made to develop relatively rapid, inexpensive and reproducible methods that can be used either in the development of new dosage forms or in the quality control of existing products. These attempts have usually been concerned with the development and use of in vitro models that simulate and describe the dissolution and absorption of drugs in vivo. Such models have allowed many useful studies to be carried out on the effects of a variety of factors that are important in the design and control of drug properties. Examples of such studies can be listed as follows:-

(a) Effects of physicochemical properties of drugs on their dissolution rates (Nelson, 1962a; Nelson et al, 1962; Higuchi et al, 1965; Wuster and Taylor, 1965).

(b) Effects of manufacturing processes, excipients and tablet coating on the release of drugs from dosage forms (Levy and Hayes, 1960; Levy et al, 1963; Morrison and Campbell, 1965; Paikoff and Drumm, 1965; Wood, 1965).

(c) Screening potential dosage forms for bioavailability purposes (Yen, 1964).

(d) Retrospective studies carried out in attempts to explain the clinical failure of a particular dosage form (Keller, 1960; Campagna et al, 1963; Levy, 1964).

(e) Sensitive quality control procedures aimed at the detection of changes in drug release characteristics caused by batch-to-batch variation, formulation changes or storage conditions. (These changes may or may not be detected by a less sensitive in vivo method.)(Markus, 1970; Pernarowski, 1970).

(f) Indication of differences in the in vivo absorption characteristics of drugs and provision of a secondary standard to detect dosage forms with a potential for poor bioavailability (U.S.P.XVIII; U.S.N.F.XVIII).

The use of in vitro dissolution methods as quality control procedures in the latter two examples have somewhat different requirements from each other. When they are used as a quality control screening procedure, as in (e),to detect batch-to-batch differences or changes in the dissolution characteristics during storage of dosage forms the principal requirement will be the sensitivity and reproducibility of the method to detect small differences. However, these differences may or may not be indicative of differences in in vivo bioavailability that are the subject of (f).

There are probably at least 100 or more different types of apparatus that have been proposed for the determination of the dissolution rates of drugs themselves or drugs from dosage forms. Classifications of these types.have been given by Hersey (1969) and Swarbrick(1970) and the factors that affect the sensitivities of the different methods and extents of correlations between in vitro and

in vivo parameters have been reviewed by Wagner (1961); Higuchi (1967); Wood (1967); Fincher (1968); Levy (1970); Gibaldi (1977c) and Nelson and Miller (1979).

1.2.3 Correlation between in vivo and in vitro data

The significance of the in vitro dissolution testing should never be overlooked or taken for granted, since the in vitro release or dissolution rates do not necessarily reflect in vivo absorption rates. The in vitro tests of any sort have no intrinsic value per se but are useful only to the extent that they correlate with quantitative in vivo results. This approach can only be achieved when the in vitro tests are able to simulate in vivo conditions (Filleborn, 1948).

While there are reports in the literature describing correlations between in vitro disintegration and dissolution times and in vivo availability, there are also many other reports in which such correlation is not observed (Morrison and Campbell,1965; Wagner, 1971b).

The limitations associated with in vitro dissolution tests reflect the complex physiological mechanisms involved in drug release and absorption. Furthermore, it is not generally appreciated that components in a formulation may themselves alter physiological mechanisms involved in the determination of bioavailability. These in vivo factors can mask or distort possible correlations. Currently used dissolution tests do not predict the effect of these physiological changes. New types of oral dosage forms, which involve the use of osmotic pressure elevators or oily vehicles,can alter significantly some of the physiological functions which cannot be

detected by *in vitro* tests. These could include variable gastric emptying and intestinal rates or changes in GI pH. Dissolution tests may indicate the type of the release, but *in vivo* performance can only be determined by *in vivo* measurement in man.

However, where physiological factors play no significant role in the absorption process, *in vitro* dissolution tests do have a significant predictive value for drugs that exhibit dissolution rate limited absorption. Many reports show variations in clinical response between two or more orally administered dosage forms containing chemically equivalent amounts of a drug, usually of limited aqueous solubility. These have been summarised by Barr (1969) and Riegelman (1969). Frequently, this variation has been traced to differences in the dissolution rate of the drug from the dosage form, which, in turn, affected the GI absorption of the drug. Thus, solid dosage forms (e.g. tablets) exhibiting inadequate clinical activity possessed a low dissolution rate relative to those tablets giving the expected response or activity. Tablets having an enhanced availability or pharmacologic response invariably had a rapid rate of dissolution.

Wagner (1971c) classified *in vivo* - *in vitro* correlations into two types:-

(i) Quantitative correlations where the *in vivo* variable (Y) is related to the *in vitro* variable (X) by an equation such as $Y = bX$, $Y = a + bX$, $\log Y = \log Y_o - bX$, $Y = a + \log X$, etc. These are obviously the more informative correlations. However, such a relationship should probably be derived only when there is a theoretical reason for relating the variables as indicated by the equation derived. For example, Bates et al (1969) found a correlation between the mean cumulative percentages of

salicylamide (Y) excreted in the urine one hour after administration of these dosage forms of the drug and the percentage of salicylamide dissolved after 15 minutes in vitro (X).The correlation could be expressed as $Y = 0.47 X + 0.07$. A relation $Y = - 0.544 + 1.003 \log X$ was established by Maeda et al (1979) for three griseofulvin dosage forms. In this relationship Y represents the mean plasma level of the drug and X is the amount of drug that is released from the dosage form within 30 minutes in an in vitro dissolution tests.

(ii) Rank order correlations, in which (a) Y increases as X increases, (b) Y increases as X decreases or (c) Y decreases as X increases. For example, MacDonald et al (1969) found a rank order correlation between the AUCs for four tetracycline hydrochloride dosage forms and the corresponding dissolution half-lives of the drug from the dosage forms. The higher the dissolution half-life the lower the AUC. In addition, a direct rank order correlation was observed between the amount of salicylate excreted in the urine after one hour following administration of four aspirin dosage forms and the amount of aspirin that dissolved in 10 minutes in vitro (Levy et al, 1961).

Some variables derived from in vivo data that have been correlated with variables derived from in vitro are:

peak blood levels,

area under the blood level curve between time 0 and t,

absorption rate constant,

amount of drug excreted in the urine in a given time,

urinary excretion rates at given times, and

pharmacological responses such as blood sugar lowering, blood pressure, pain relief, etc.

Similarly, variables derived from *in vitro* data that have been correlated with *in vivo* data include:

disintegration time,

time for some percentage of the drug to dissolve in vitro, e.g. $t_{50\%}$ - time for 50% of the drug to dissolve,

concentration of solution or amount in solution at a given time,

intrinsic dissolution rate, and

first order rate constant of the dissolution process (Wagner, 1971c).

The correlation of *in vivo* with *in vitro* data has been reviewed extensively by Levy (1966), Swarbrick (1970), Wagner (1971c) and Barr (1972).

1.3 Physicochemical factors affecting drug absorption

1.3.1 pH - partition theory

The dissociation constant and lipid solubility of a drug, as well as the pH at the absorption site, often dictate its absorption characteristics. The interrelationships among these parameters are known as the pH - partition theory of drug absorption (Gibaldi, 1977c; Poole, 1979).

The salient points of this theory are (a) that the epithelium of the GI tract and other biological membranes act as lipid barriers, which separate aqueous phases;(b) drugs pass from one aqueous phase to another by a process that involves partition between one of those phases and the membrane, diffusion across the latter and finally a

second partition between the membrane and the other aqueous phase; (c) since partition is of such importance in the transport process the membrane:aqueous phase partition coefficient of the drug will also be important; (d) many drugs are weak electrolytes and consequently will ionize in aqueous solution. The degree of ionization will depend on the pKa value of the particular drug and the pH of the aqueous phase in accordance with the Henderson-Hasselbalch equations for weakly acidic and weakly basic drugs (Eq.1.1 and 1.2, respectively).

$$pKa - pH = \log \left(\frac{\text{unionized acid}}{\text{ionized acid}} \right) \qquad \text{(Eq. 1.1)}$$

$$pKa - pH = \log \left(\frac{\text{ionized base}}{\text{unionized base}} \right) \qquad \text{(Eq. 1.2)}$$

(e) since the unionized form of a drug will exhibit a higher lipid membrane:water partition coefficient it follows from (c) above that ease of transport <u>in vivo</u> via passive diffusion will be markedly affected by the pKa value of the drug and the pH of the aqueous phase, into which, or from which, partition is occurring.

Many investigators (e.g. Travell, 1940; Shore et al,1957; Schanker et al, 1957 and 1958; Hogben et al, 1957 and 1959; Schanker, 1960; Kakemi et al, 1967; Doluisio et al, 1969a) have substantiated the pH - partition theory as indicated in the review by Levine (1971). However, application of the theory suggests that weakly acidic drugs would be mainly absorbed from the stomach whilst weakly basic drugs would be preferentially absorbed from the intestine. The extremely larger surface area of the small intestine, compared with the stomach, more than compensates for the effects indicated by the pH - partition theory, and consequently, the small intestine tends

to be regarded as the major site of absorption for all drugs (Levine, 1971) (see part 1.4.3a of this Chapter). However, the stomach still functions as an absorptive site for weakly acidic drugs. For example, Nayak and Benet (1974) showed that although salicylic acid is more rapidly absorbed from the intestine than from the stomach in unanaesthetised rhesus monkeys the extent of absorption appeared to be the same from both regions of the GI tract. Thus, it seems reasonable to suggest that the contribution of absorption from the stomach to the total amount of absorption should not be discounted, particularly for weakly acidic drugs when gastric emptying rate is delayed. The rate of stomach emptying will, in fact, have a marked effect on the rate of drug absorption because this emptying rate will govern the rate of appearance of drug at the major absorption site, i.e. in the small intestine (see part 1.4.1a of this Chapter).

1.3.2 Drug dissolution and release

Drugs administered orally in solid dosage forms, such as tablets, capsules or suspensions, are not immediately available to the body, since they are normally absorbed from solution (Morrison and Campbell 1965; Cadwallader, 1974). This means that such drugs must dissolve in the GI fluids before absorption occurs. A schematic illustration of the important processes involved following oral administration of dosage forms containing solid drug, i.e. tablets, capsules and suspensions, is given below:-

Drug in solid dosage form → (i.e. dissolution) Drug release → Drug in solution at absorption site → (Absorption) Transport from GI tract to blood → Drug in systemic circulation.

Either dissolution or absorption may be the rate-limiting stage in the overall process. When dissolution is the controlling step absorption is said to be dissolution rate limited. Dissolution rate limited absorption may occur even if the drug is given as a solution of a water soluble salt. For example, a sodium salt may be precipitated as the free acid in the gastric juice (Munzel, 1971) but in the form of very fine particles, which favour rapid dissolution. Since dissolution precedes absorption, any factor affecting the rate of solution also must affect the rate of absorption. Consequently, dissolution rate may affect the onset, intensity and duration of clinical response.

A general relationship describing the dissolution process was first observed by Noyes and Whitney (1897). The Noyes-Whitney equation states that:

$$\frac{dC}{dt} = kS \quad (C_s - C_t) \qquad \text{Eq. 1.3}$$

where dC/dt is the dissolution rate, k is a constant, S is the surface area of the dissolving solid, C_s is the concentration of drug in the diffusion layer surrounding the solid material and C_t is the concentration of the material in the solvent at time t. The constant k has been shown to be equal to D/h (Higuchi, 1967), where D is the diffusion coefficient of the dissolving material and h is the thickness of the diffusion layer. The diffusion layer is , a thin, stationary film of solution encapsulating the surface of the solid. It is saturated with the drug and hence C_s may be equated with the solubility of the drug in the solvent. The term $(C_s - C_t)$ in Eq. 1.3 represents the concentration gradient between the diffusion layer and the bulk solution. In dissolution-rate limited absorption,

C_t is negligible compared to C_s, i.e. so called sink conditions exist, and Eq. 1.3 may then be written as:

$$\frac{dC}{dt} = \frac{D\ S\ C_s}{h} \qquad \text{Eq. 1.4}$$

Eq. 1.4 describes a diffusion controlled dissolution process. It is envisaged that when the solid is introduced to the dissolution medium, the drug rapidly saturates the volume of liquid immediately adjacent to the surface and thereby creates the diffusion layer. Drug molecules diffuse from the saturated layer to the bulk (the slow step in the dissolution process) but are immediately replaced in the diffusion layer by molecules from the solid surface. This is known as the diffusion layer model of dissolution. On the other hand, when diffusion from the saturated layer to the bulk is relatively rapid and the reaction at the solid/liquid interface is not instantaneous then this reaction may become the rate limiting step with respect to the mass transport process. This is known as the interfacial barrier model of dissolution.

Eq. 1.4 is an oversimplified representation of the dynamics of dissolution. Nevertheless it is qualitatively useful and permits a consideration of the effects of many factors on dissolution rate. The solubility (C_s) of many drugs increases with increasing temperature and is influenced by minor changes in chemical structure (e.g. salt versus parent acid or base), crystal structure, degree of solvation and interaction with other ingredients in a dosage form. On the other hand, the diffusion coefficient (D) is inversely related to viscosity, and dissolution rate decreases as the viscosity of the solvent increases. Increasing the surface area (S) of drug exposed to the

dissolution medium, by reducing the particle size or by any other means, frequently increases the dissolution rate. Changes in the above parameters caused by formulation factors or manufacturing techniques are obviously important from a bioavailability point of view. Consequently, numerous review and research articles concerned with dissolution rate - limited absorption and the factors affecting dissolution rates and bioavailabilities of drugs have appeared in the literature. Some of these are listed below: Nelson (1962b), Delgado and Cosgrove (1963), Poole (1969), Schneller (1970), Munzel (1971), Monkhouse and Lach (1972), Ritschel (1973), Greenblatt et al (1974), Yamamoto et al (1974), Haleblian and Goodhart (1975), Goodhart and Eichman (1976), Gibaldi (1977c), Nayak et al (1977), Maeda et al (1979), Mathur et al (1979), Poole (1979), Resetarits et al (1979), Wade (1980) and Notari (1980b).

Occasionally the choice of formulating a sparingly water soluble drug as an aqueous suspension or as a solution or a suspension in a non-aqueous organic solvent arises. However, the choice of vehicle can affect the bioavailability of the drug. Thus, drugs dissolved in non-aqueous solvents that are miscible with biological fluids, e.g. glycerin, are usually rapidly absorbed when compared with solid dosage forms of any type, since the very fine particles precipitated after dilution with the biological fluids favour rapid dissolution rate.

In the case of drugs that are dissolved or suspended in water-immiscible liquids, e.g. an oily vehicle, then an additional phase or compartment must be included in any model of the release process. In fact, the release of drug particles from such media in vivo may become the rate limiting step in the overall absorption process.

(The physiological effects of an oily vehicle may also have a considerable affect on the bioavailability of the drug - see Chapters2 and 3 of this Section).

The mechanisms involved in the release of solid drugs from this type of suspension are complex and detailed information on these mechanisms with respect to orally administered oily suspensions is not available. However, in vivo and in vitro bioavailability studies on fatty suppositories containing suspended drugs (Bevernage and Polderman, 1973; Schoonen et al, 1976 and 1979; de Blaey and Rutten-Kingma, 1977; Rutten-Kingma et al, 1979a,b,c and d) and associated in vitro studies on drugs suspended in liquid paraffin (Crommelin, 1980a and b; Crommelin and de Blaey, 1980a and b) indicate that the following mechanisms of drug release may operate.

(a) Drug that is dissolved in the oily vehicle is released by a process that involves diffusion through the water immiscible vehicle and partition at the interface into a surrounding aqueous body fluid.

(b) If the drug is suspended in the water immiscible vehicle then the release process involves movement of drug particles through the vehicle to the interface followed either by dissolution at the interface into the surrounding aqueous phase, or by complete passage of particles through the interface into the aqueous phase, in which dissolution then occurs.

The previously quoted studies show that the relative importance of these mechanisms and of each process within them depends on factors such as the concentration of drug in the non-aqueous vehicle, its solubility in this vehicle and in water, the particle size of the suspended particles and the viscosity of the vehicle. For example,

in the case of compounds that are insoluble in the non-aqueous phase but readily soluble in water, such as sodium chloride,sodium salicylate and sodium phenobarbitone, the release was shown to be controlled by the rate of transfer of the solid particles through the oily vehicle to the interface, since the rates of other processes, e.g. dissolution in water, are rapid. In contrast, when less water soluble compounds are used, e.g. paracetamol and chloramphenicol, then mass transfer to the interface occurs at a faster rate than dissolution into the aqueous phase and this later stage is consequently the rate determining one for the release process.

Although these studies on the bioavailability of drugs suspended in fatty suppository bases provide a lot of useful information there are several other factors that may affect the release process and that still require investigation, e.g. the effect of gut motility on the thickness of layers of water immiscible vehicles and on the kinetics of particle movement within those layers, and the effect of pH on the overall release process because the non-aqueous vehicle may act as a reservoir for parent acids or bases that may be formed when a water soluble salt is released from the vehicle into the GI fluids.

1.4 Physiological factors affecting absorption

1.4.1 Factors affecting transit to the site of absorption

(a) Gastric emptying rate (GER)

The importance of gastric emptying with regard to drug absorption is readily apparent in the light of the pH-partition theory. Delay in the gastric emptying, caused by any factor, will be

a particularly important determinant of drug absorption in the following situations:

(1) Where the absorption of the drug is favoured by the acidic environment of the stomach.

(2) Where drugs are optimally absorbed from the small intestine.

In the first category, according to the pH-partition theory, acidic drugs are absorbed well from the stomach. Although the surface area available for absorption from this organ is small compared to that of the small intestine, the extent of salicylic acid absorption appeared to be identical from both regions of the rhesus monkeys tract (Nayak and Benet, 1974). Significant gastric absorption of aspirin was also reported by Truitt and Morgan (1960 and 1964) and Saunders (1974a)has stated that acetylsalicylic and salicylic acids are absorbed rapidly in the stomach. It appears, therefore, that an increase in the time that such drugs reside in the stomach (delay in gastric emptying rate) would lead to an increase in the contribution that such absorption makes to the overall extent of GI absorption. In addition, the slower release of drug from the stomach may improve the efficiency of absorption from the intestine or allow a longer period for drug dissolution to occur before transfer into the intestine. Various examples of the enhancement of absorption of weak acidic drugs that can be ascribed to delays in the GER have been reported. Peterson and Finland (1942) found that the absorption of sulphadiazine is more complete, although slower, when it is given after a meal than if it is given on a fasting stomach in man. Fatty meals reduced the rate but increased the extent of aspirin absorption (Koch et al, 1978). The bioavailability of phenytoin was increased markedly when coadministered with food (Melander et al, 1979). A more complete

absorption, although slower, was found when furosemide was administered after a meal than in the absence of food in the rat (Chungi et al, 1979). A significant increase in the amount of drug absorbed, but not in the rate of absorption, occurred when aspirin and salicylic acid were administered in aqueous suspensions with different viscosities to the rabbit (Barzegar-Jalali and Richards, 1979b).In fact a linear relationship was observed between the log of apparent viscosity of the suspending medium at a shear rate of 100 s^{-1} and 37^{o}C and the amount of drug absorbed in 9 hr. Cook and Hunt (1970) showed that aspirin absorption was decreased ten times in situations where the rate of gastric emptying was increased by alkalinization the stomach medium.

In the second category, which applies to the majority of drugs, absorption is optimal in the intestine because of (i) the physico-chemical properties of the drug and the pH environment of this region of the tract (e.g. weakly basic drugs), (ii) the presence within the intestinal mucosa of "carrier" molecules, which are required for the active or facilitated transport of drugs, (iii) the use of enteric coated dosage forms, which are formulated so as to prevent drug release in the stomach fluid but to allow rapid drug release in the mildly acid fluids of the duodenum or (iv) the large total absorptive epithelial surface area of the intestinal mucosa. A delay in the rate at which drugs in this category leave the stomach and enter the duodenum may have a pronounced effect on the onset of therapeutic effect, the overall rate of drug absorption, the intensity of effect, and, occasionally, the biological availability. The process of gastric emptying is also of importance when considering drugs that are prone

to chemical degradation in the stomach.

The terms most frequently used in the literature to quantitate emptying are emptying time, emptying half-life and emptying rate. Emptying time generally refers to the time needed for the total contents initially present to leave the stomach, emptying half-life is the time needed for the stomach to empty one half of its initial contents and emptying rate refers to the speed with which stomach contents leave the stomach. Note that there is an inverse relationship between emptying time or half-time and emptying rate.

It has been suggested that gastric emptying takes place by a monoexponential (i.e. first order) kinetic process (Hopkins, 1966; Hunt and Knox, 1968a). As a result, a semilogarithmic plot of the volume of a liquid meal or dosage form remaining in the stomach versus time will provide a straight line relationship, from the slope of which the rate constant associated with emptying can be derived. However, Wagner (1971d) pointed out that small amounts of liquids appear to empty from the stomach at essentially constant (zero order) rates whilst larger volumes obey the first order process as mentioned above.

GER is influenced by a variety of factors. The composition and viscosity of a meal ingested by a subject may significantly influence the rate of gastric emptying. For example, fats (Davenport, 1971a and b), in any form, not only inhibit gastric secretion but have a considerable inhibitory effect on gastric emptying.(See Chapter 2 in this Section for information on the mechanism of action of fats.) Proteins and starch also inhibit gastric emptying but Bachrach (1959a) suggested that their effects are less pronounced than those produced by fat.

However, Hunt and Stubbs (1975) reported that isocaloric concentrations of triglycerides and carbohydrate gave equal slowing of gastric emptying. For example, 4g triglycerides/100 cm^3 meal slowed gastric emptying to the same extent as 9g carbohydrate/100 cm^3 meal and both meals were equivalent to 36 Kcal/100 cm^3. Amino acids reduce the rate of gastric emptying to an extent directly dependent upon concentration, probably as a result of osmotic pressure (Cook and Moulang, 1972).

Hypertonic solutions are emptied more slowly than pure water or hypotonic solutions.(See Chapter 2 in this Section for information on the mechanism of action of hypertonic solutions.) For example, the absorption of drugs administered in a concentrated sucrose solution was decreased in rabbit and rat, due to delay in the GER (Kato et al, 1969). Since all the drugs, i.e. aminopyrine, dipyrone, phenobarbitone and strychnine, that were investigated by Kato et al are absorbed primarily from the intestine, the gastric emptying hypothesis fits their observations. However, Hem (1973) commented that "unfortunately, these workers did not study a drug that is absorbed chiefly from the stomach. An increased relative availability from such a drug in the sucrose vehicle would be strong support for their hypothesis". Malone et al (1960) reported that addition of sucrose to the vehicle is responsible for the delayed absorption pattern of phenobarbitone in the rat. The temperature of the meal may be critical in the rate of gastric emptying. Davenport (1971b) has indicated that cold meals increase and hot meals decrease the emptying of gastric contents. The latter phenomena is perhaps an example of a physiological protective action that occurs to prevent possible damage to the intestinal mucosa.

The viscosity of the ingested meal or that of a liquid dosage form of a drug may also affect the emptying of stomach contents. Usually, as the viscosity of the gastric fluids is increased, there is a corresponding decrease in the rate of emptying (Levy and Jusko, 1965). Enhancement of absorption of riboflavin occurred when administered in a high viscosity(2%w/v)sodium alginate solution (Levy and Rao, 1972). On the other hand, increases in the viscosity of aqueous vehicles used for nitrofurantoin suspensions led to decreases in the rate (Seager, 1968; Soci and Parrott, 1980) and extent of absorption (Seager, 1968) in man and in the extent of absorption in the rat (Barzegar-jalali and Richards, 1980).

Gastric emptying may also be influenced by the positioning of the individual. In a person lying on his left side gastric emptying is reduced because the natural curvature of the gastric pouch gives rise to an uphill path leading to the duodenum. However, if the subject is lying on his right side, emptying is facilitated (Bachrach, 1959b). Significant differences may be observed in the onset of therapeutic effect depending on whether a patient is ambulatory or bedridden. The emotional state of the patient also may influence the stomach motility. Aggressive or stressful emotional states increase stomach contractions and emptying rate whereas depression reduces the rate (Bachrach, 1959b; Almay, 1973).

The higher acidity of the duodenal contents, the slower the GER (Hunt and Knox, 1969). Lower molecular weight acids are more effective than those of higher molecular weight. Low concentrations of sodium bicarbonate (e.g. 1%) increased the rate of emptying while higher concentration decreased it (Shay and Gershon-Cohen, 1934).

Fluids or suspensions of small particles empty more rapidly than chunks of material (e.g. large granules or tablets), which must first be reduced in size prior to emptying (Davenport, 1971a; Wagner, 1971c). Intact tablets have been observed in the stomach as long as 6 hr after ingestion of an enteric-coated product with a meal (Blythe et al, 1959). Gastric emptying is one of the more important factors contributing to the usually large intersubject variability in the absorption of drugs from enteric-coated tablets. As a means of reducing this variability, it has been suggested that enteric-coated medication be administered in the form of small, individually coated granules that would empty gradually but continuously into the duodenum (Wagner et al, 1960).

Differences in gastric emptying among subjects also contribute to the variability in absorption rate of drugs from conventional dosage forms. For example, after administration of 1.5g (3 tablets) of paracetamol to 14 convalescent hospital patients, the maximum concentration in the plasma ranged from 7.4 to 37.0 $\mu g/cm^3$, and the time required to reach the maximum concentration ranged from 30 to 180 minutes(Heading et al, 1973). Both these indices of absorption rate were linearly related to the gastric emptying half-life found in each patient.

Gastric emptying can be prolonged or prompted significantly by a number of drugs. For example, the anticholinergic drug propantheline significantly reduces the rate of absorption of riboflavin and enhances its extent of absorption (Levy et al, 1972). Metoclopramide, on the other hand, significantly increases the absorption rate of ethanol (Gibbons and Lant, 1975), tetracycline

and paracetamol (Nimmo, 1973) and the rate of extent of absorption of L-dopa (Wade et al, 1974; Mearrick et al, 1974).

GER is reduced by bile salts (Menguy, 1960; Hunt, 1975). These salts were reported to inhibit markedly gastric emptying as well as proximal intestinal transit in the rat (Feldman and Gibaldi, 1968; Feldman et al, 1968), and it was suggested that these findings would be similar in man (Mayersohn et al, 1969).

(b) Intestinal transit

Once a dosage form empties from the stomach and enters the small intestine, it will be exposed to an environment totally different from the stomach. Since the small intestine is the primary site of drug absorption, the longer the residence time in this region the greater the potential for efficient absorption, assuming the drug is stable in the intestinal fluids and will not react with endogenous material to form water-insoluble derivatives.

There are primarily two types of intestinal movements, propulsive and mixing. Propulsive movements, generally synonymous with peristalsis, propel intestinal contents down the tract at about 1-2 cm/sec (Guyton, 1971). Thus, under normal circumstances, movement down the tract is relatively slow and it takes 3-10 hr to move a meal in the form of chyme from the pylorus to the ileocaecal valve (Guyton, 1971). These propulsive movements will primarily determine intestinal transit rate and, therefore, the residence time of a drug in the intestine. This time of residence is important since it will dictate the amount of time available for the dosage form to release the drug, permit dissolution, and allow for absorption. Both release and mucosal permeation, stated Hayton (1980), must occur before gastrointestinal transit removes the drug from sites of absorption that are located

primarily in the small intestine and particularly in the proximal region. For a drug to be considered well absorbed by the oral route, release and permeation must occur quickly relative to GI transit.

Mixing movements of the small intestine are a result of contractions dividing a given region of the intestine into segments producing an appearance similar to a chain of sausages. These contractions result in the mixing of the intestinal contents with secretions several times a minute. These movements bring the gut contents into intimate contact with the surface epithelium and thereby provide a large effective area for absorption. This contact and agitation provided by the peristalsis waves facilitate absorption by reducing the mean free diffusion path of the drug molecules to the intestinal mucosa.

Obviously, the slower the intestinal motility, the longer the residence time and the more complete may be the process of dissolution and absorption. Excessive peristalsis (Propulsive) or intestinal motility would be expected to produce the opposite effect.

Intestinal motility will be more important for those dosage forms that release drugsslowly (e.g. sustained-release products), require time to initiate release (e.g. enteric-coated tablets), or contain drugs that are dissolved slowly or are absorbed by a specialised mechanism only in a certain region of the intestine.

Propantheline and similar drugs significantly increase transit time, whereas metoclopramide accelerates transit through the small intestine. The extent of absorption of drugs that are incompletely absorbed may be dependent on intestinal motility. Enhancement of riboflavin absorption in man by preadministration of propantheline (Levy et al, 1972) or by administration of the vitamin in a highly

viscous sodium alginate solution (Levy and Rao, 1972) has been reported. Retention of the vitamin at its specialised absorption sites for prolonged time periods because of the increase in the GI transit time, brought about by the anticholinergic agent or by the high viscosity, was proposed as the rationale for the absorption data. Enhancement of the absorption of poorly water soluble drugs, such as digoxin (Manninen et al, 1973) and phenolsulphonphthalein (Ashly and Levy, 1973) by preadministration of propantheline has also been observed. However, decrease in the absorption of digoxin was demonstrated in patients receiving metoclopramide (Manninen et al, 1973). These workers suggested that the increased residence time at the site of absorption permitted a better dissolution and absorption of digoxin.

(c) Recycling processes

Once absorbed, certain drugs are returned to the GI lumen by way of the stomach, bile, or intestine and are then available for subsequent reabsorption. These recycling processes may complicate the assessment of bioavailability by appearing to extend the time necessary for absorption of the administered drug by producing an erratic plasma concentration-time curve.

Enterogastric and enterointestinal recycling processes are not particularly important. Nevertheless, because both processes ensure an essentially continuous flux of drug between blood and GI contents, as viewed from the blood, the GI tract may be regarded as just another equilibrating tissue, As such, the GI tract will be evident in the disposition kinetics of a drug given intravenously and accordingly forms part of its volume of distribution.

Enterohepatic recycling potentially offers the greatest problem. Earlier comments about blood flow and volume of distribution are still applicable, and although the liver receives 25% of the cardiac output (Rowland, 1973), accumulation in bile only becomes quantitatively important for drugs that are extensively cleared by the liver into bile and that possess a relatively small volume of distribution.

A quantitative kinetic description of enterohepatic recycling is difficult. Any bile formed is stored and concentrated within the gall bladder in man and some animals. Stimuli, especially food and fats, enhance the evacuation of bile from the gall bladder (see parts 1.4.2 b and d of this Chapter) but this evacuation occurs in a discontinuous manner, which complicates the picture. If the fraction of the dose in the bile is sufficiently great and subsequent reabsorption rapid, secondary peaks may appear in the blood concentration-time curve. A drug, or more usually a drug conjugate, is excreted into the bile and enters the GI tract where, in the case of the metabolite, it may be broken down by enzymes in the gut or gut flora to liberate the unchanged, parent drug (Williams et al, 1965; Plaa, 1975; Routledge and Shand, 1979). Any drug appearing in this way may then be reabsorbed into the body as well as any drug which may have appeared in the gut from a recently taken oral dose. This might be responsible for the prolonged retention of certain drugs and drug metabolites in the body (Williams et al, 1965).

1.4.2 Effect of constituents of the GI fluids

(a) Hydrogen ion concentration and enzymes

pH varies considerably along the length of the GI tract and may have an important influence on drug absorption. Not only is it important because it determines the degree of ionization of the drug, but it is also an important determinant of the degree of solubility, and hence dissolution, of poorly water soluble drugs and of the degree of drug degradation that may occur in the GI tract.

In man, the usual pH range of gastric fluid is 1-3, whilst intestinal fluids range from approximately 5-6 in the duodenum to 7-8 in the proximal jejunum and approach a pH of about 8 in the large intestine (Borgstrom et al, 1957; Wagner, 1961).

Differences in the pH along the GI tract and any changes in this pH, that may be caused by several factors, will affect the absorption of weakly organic acidic or basic drugs because of changes in the extent of ionization, since the unionized moiety of the drug is absorbed preferentially as indicated in part 1.3.1 of this Chapter.

The pH of gastric fluid is subject to a great deal of variation. Gastric secretions have a pH of less than 1, but the pH of gastric contents is usually between 1 and 3 because of dilution and diet. The pH of the stomach contents is distinctly but briefly elevated after meals; pH values of 5 are not unusual (Gibaldi, 1977b). Fasting tends to decrease the pH of gastric fluid to 1.2 - 1.8 (Martin, 1955). Disease may also influence the pH in the stomach (James and Pickering, 1949). Fats and fatty acids have been found to inhibit gastric secretion (Menguy, 1959a; Johnson and Grossman, 1969; Christiansen et al, 1976). A major clinical effect of antispasmodic drugs, such as atropine and propantheline, is a reduction in gastric

secretion. Some anticholinergic activity, including suppression of secretion, is commonly found with many other drugs. A large number of antacid products are widely used for the purpose of neutralising gastric acidity and elevating the pH of gastric contents (Hurwitz, 1977).

These factors may significantly influence the gastric absorption of weakly acidic drugs which are usually efficiently absorbed at the normal low pH of the gastric fluids. However, Pottage et al (1974), reported that the rate, but not the extent, of absorption of aspirin is significantly higher in achlorhydric patients than in normal ones, due to the higher solubility of the drug in the less acidic gastric fluid.

The pH of the GI fluids may also be an important factor when considering the stabilities of drugs in the GI tract. In fact the lack of oral activity of certain drugs, when compared to their activities following parenteral administration, can be attributed to some type of degradative reaction that is catalyzed by the high acidity and enzymatic activity of gastric fluids. The end products of such reaction usually possess no activity. Some examples of drugs prone to acid catalyzed degradation are certain penicillin derivatives (Broderson, 1949) and erythromycin and its esters (Stephens et al, 1959; Nelson 1962a).

Drug inactivation in gastric fluid competes with stomach emptying and absorption as processes of drug removal from the stomach, once solution of the drug has been achieved. That portion of a dose, which is emptied into the intestine in an undissolved state, can be expected to become available for intestinal absorption.

Since GI fluids contain various enzymes essential for the digestion of food, it is not unreasonable to expect that these enzymes may be responsible for the metabolism of certain drugs. More details of this aspect will be discussed later in this chapter (see 1.4.3d).

(b) Bile salts

Bile salts, which are physiological surface active agents, may enhance the rate and extent of absorption of poorly water-soluble drugs by the following mechanisms:

(i) Increased drug dissolution rates have been noted in studies in vitro in the presence of bile salts (Bates et al, 1966a; Weintraub and Gibaldi, 1969) and in the presence of lysolecithin, another naturally occurring surface active agent found in duodenal fluid (Bates et al, 1967).

(ii) Inhibition of gastric emptying and proximal intestinal transit and alteration of membrane permeability were the reasons suggested by Mayersohn et al (1969) who observed enhancement in the absorption of riboflavin and flavin mono-nucleotide when bile salt was given prior to the drug. In addition to their effect on GER, bile salts are known to alter membrane permeability (Davenport, 1968; Feldman and Gibaldi, 1968; Feldman et al, 1968; Hori et al, 1977; Richards and Gardner, 1978). Significant increase in the permeability of the everted intestine to salicylate by bile salt has been reported (Feldman and Gibaldi, 1969a and b).

The increased absorption of many drugs administered after a fatty meal or together with an oil may be caused not only by the resultant delay in GER, but also by the fact that bile is secreted into the small intestine in response to the presence of fats (Ivy,

1934; Sjovall, 1959; Bates et al, 1966b; Bates and Gibaldi, 1970; Bates and Sequeira, 1975; Bates et al, 1977).

Cavallito and O'Dell (1958) have shown that cholic acid (a natural bile acid found in man) and dehydrocholic acid (a synthetic bile acid derivative) increase the intestinal absorption of several quaternary ammonium hypotensive agents in dog as judged from blood pressure lowering measurements. These steroidal acids may enhance absorption of quaternary ammonium compounds by increasing membrane permeability, by mucolytic action which reduces the barrier effects of intestinal mucins, by forming complex salts having a greater ability to penetrate the membrane, or by increasing bile secretion which will enhance drug dissolution.

Based on these findings it is suggested that bile salts, normally present in the small intestine, enhance the absorption of poorly water-soluble drugs. It follows that in those conditions where there is a diminished bile salt concentration in the proximal intestine, such drugs will be poorly absorbed. This possibility is supported by the findings of Pekanmaki and Salmi (1961), who found that absorption of phenolphthalein is markedly decreased when bile drainage into the intestine is prevented in the cat.

Bile salts, on the other hand, have been found to form insoluble, non-absorbable complexes with such drugs as tubocurarine (Mahfouz, 1949) and neomycin and kanamycin (Faloon et al, 1966).

(c) Mucin

Mucin, a viscous mucopolysaccharide, which lines and protects the gastric and intestinal surface epithelium, may retard drug absorption by acting as an effective barrier to diffusion or by forming non-

absorbable complexes with some drugs, e.g. quaternary ammonium compounds (Levine et al, 1955; Levine and Pilikan, 1961). With respect to this observation, a study cited above (Cavallito and O'Dell, 1958) indicated that certain bile acids may potentiate the absorption of quaternary ammonium hypotensive agents. A possible mechanism would be the mucolytic action of bile acids, which might reduce the barrier effect of mucin or conceivably alter the binding of quaternary ammonium compounds to mucin.

Tetracycline has been shown to strongly bind to hog gastric mucin (Saggers and Lawson, 1966) and other workers have shown reduced tetracycline absorption in the perfused rat intestine in the presence of added mucin (Braybrooks et al, 1975) and reduced tetracycline dissolution rate in vitro in the presence of mucin (Kellaway and Marriot, 1975). It has also been noted (Abbott et al, 1959) that the disintegration time for tablets is longer in human gastric fluid containing mucin compared to the times seen in simulated gastric fluid. Hunter et al (1980) found that rapidly disintegrating hard gelatin capsules when administered with a small amount of liquid to fasting subjects did not disperse in the stomach compared with non fasting subjects. A possible explanation was given that adherence to the viscous mucin retards the dispersion process. The capsule shell may become coated with gastric mucin and may not dissolve for a longer time. As a result, the drug particles may become coated with a gelatinous film, which retards dispersion and dissolution (Anon, 1972).

(d) Food

Any factor that influences drug bioavailability may do so by altering the rate and/or extent of absorption. The following mechanisms

appear to be the primary methods whereby food and diet may alter drug bioavailability:

(i) Changes in the gastric emptying and intestinal transit rates.

(ii) Food-induced secretion of GI fluids, which may act on the drug molecule.

(iii) Physicochemical properties of the drug and composition of food.

(iv) Food may decrease the amount of biological fluids available to the drug, thereby decreasing the dissolution rates of solid dosage forms.

(v) Food may increase the viscosity of the medium and decrease the rate of drug diffusion to the mucosal barrier.

(vi) Food components may compete with the drug for absorption.

(v) Food components may adsorb or interact with a fraction of the dose and reduce the amount of drug available for absorption.

(viii) Food components may affect metabolic transformation of drugs in the GI wall and liver.

(ix) Food, especially fatty meals, enhances the secretion of bile.

(x) The increased blood flow through the splanchnic area that occurs following a meal would increase the absorption capacity.

The literature contains numerous instances of the effects of food on drug bioavailabilities. For example, inhibitory effects have been observed with isoniazid (Melander et al, 1976a), tetracycline (Kirby et al, 1961; Neuvonen, 1976), penicillin derivatives (Klein and Finland, 1963)and rifampicin (Acocella, 1978), whilst bioavailability enhancing effects have been noted for sulphadiazine (Peterson and Finland, 1942), griseofulvin (Crounse, 1961; Kraml et al, 1962), riboflavin (Levy and Jusko, 1966), spironolactone, hydralazine and

propranolol and metoprolol (Melander et al, 1977a,b and c respectively), erythromycin stearate (Malmborg, 1978) and phenytoin (Melander et al, 1979). In other cases no consistent effects have been reported, e.g. oxazepam and metronidazol (Melander et al, 1977d and e respectively; Welling, 1980), and sulphasomidine (Melander et al, 1976b).

The complex effects of food on the absorption of drugs lead to the conclusion that the net effect on a particular drug can only be ascertained by clinical studies on that drug and should not be derived from studies on other drugs.

1.4.3 Properties of the site of absorption

(a) Surface area of the absorption sites

The major components of the GI tract are the stomach, small intestine, and large intestine or colon. The small intestine includes the duodenum, jejunum, and ileum. The major segments of the GI tract differ from one another both anatomically and morphologically, as well as with respect to their secretions and pH.

The stomach is a pouch-like structure lined with a relatively smooth epithelial surface. Extensive absorption of many weakly acidic drugs or drugs possessing physicochemical properties consistent with the permeability characteristics of the gastric mucosa can be demonstrated in the stomach under normal physiological conditions. However, under such normal conditions, when gastric emptying is not impeded, the stomach's role in drug absorption is much more modest due to limited residence of the drug and the limited surface area of the stomach.

Of all regions of the GI tract the small intestine has the greatest available surface, from which absorption can take place. This large epithelial surface area results from the existence of (i) folds in the mucosa(i.e. the folds of Kerckring or valvulae conniventes); and (ii) finger-like projections, termed villi, arising from and being part of the folds of Kerckring. Each villus is comprised of many microvilli (Levine, 1971). The irregularities in the mucosa surface, caused by the microvilli, villi, and submucosal folds, increase the area available for absorption by more than 30 times that which would be present if the small intestine were a smooth tube (Granger and Baker, 1950). Based on studies in the rat, one can estimate that the effective surface area of the small intestine is about 10 times that of the stomach (Crouthamel et al, 1971). It would be more in man, since the latter differs from the rat in having submucosal folds (Hilton, 1901). Other studies conclude that surface area decreases sharply from proximal to distal small intestine with almost half of the total mucosal area being found in the proximal quarter of the gut (Wilson, 1967). Thus, the proximal part of the small intestine has the largest capacity for absorption of most drugs as well as for most dietary constituents (Booth, 1967). The small intestine is also the most important region of the GI tract with respect to carrier-mediated transport.

Since there are no villi present in the colon, as in the stomach, the surface area is quite limited. This segment serves as a reserve area for the absorption of drugs that have escaped absorption proximally because of their physicochemical properties or their

dosage form or physiological factors, such as rapid GI transit. Only the absorptive capacity of this segment is significant when a drug is absorbed by a specialised transport process located in this area. Generally, if a significant portion of a dose of a drug by-passes the stomach and small intestine, one can anticipate a low biological availability for the drug.

(b) Local blood flow

The entire GI tract is highly vascularised and therefore well perfused by the blood stream that permits efficient delivery of absorbed material to the body. Usually, blood flow does not appear to play a primary role in drug absorption.

There are circumstances, however, where blood flow to the GI tract may influence drug absorption, e.g. when a considerable change occurs in the blood flow. A reduction in the blood flow and therefore in rate of oxygen delivery may produce a reduction in the absorption of those compounds absorbed by active transport mechanisms, e.g. phenylalanine in rats (Winne, 1973).

Absorption of freely permeable compounds through the intestinal membrane, (e.g. tritiated water, salicylic acid, and sulphaethidol) is very sensitive to blood flow. In contrast, compounds which penetrate the epithelial cells with great difficulty, e.g. ribitol, are unaffected by changes in blood perfusion. In between these two extremes are a variety of intermediate compounds (e.g. urea, methanol, etc) whose absorption rate is blood flow-limited or dependent at low flow rates, but blood flow-independent at higher flow rates (Winne, 1970; Winne and Remischovsky, 1970; Crouthamel et al, 1970; Ther and Winne, 1971).

Blood flow to the GI tract increases shortly after a meal and may last several hours. From the quantitative investigations in the rat (Reininger and Sapirstein, 1957), in the dog (Herrick et al, 1934) and in man (Brandt et al, 1955) it appears that there is approximately a 30% increase in blood flow through the splanchnic area following a meal. Digestion processes in general seem to enhance blood flow to the tract (Bynum and Jacobson, 1971). McLean et al (1978) suggested that the increase in the splanchnic blood flow, typical of those found after a meal, can have a significant effect on the availability of the drugs subject to first-pass metabolism, and postulated that such variation in the blood flow could account for the increased bioavailability of single doses of metoprolol and propranolol when administered with food compared with during the fasting state (Melander et al, 1977c).

Fasting, on the other hand, was reported to produce emotional stress (Kollar et al, 1964; Januszewicz et al, 1967), which may cause some constriction of the splanchnic vasculature and, hence, a reduction in intestinal blood flow. Reduction in the intestinal blood flow can decrease the absorption of certain drugs (Crouthamel et al, 1970 and 1975). Doluisio et al (1969b) fasted rats for periods of up to 50 hours, and subsequently performed in situ intestinal absorption rate studies with salicylic acid, barbitone, haloperidol, and chlorpromazine. They found that the rates of absorption of the test drugs were not affected by fasting until after approximately (17-20) hours. As the period of fasting extended beyond 20 hours, there was a progressive decrease in the rates of intestinal absorption of the test drugs. These investigators have proposed tentatively that

prolonged fasting may produce a decrease in blood perfusion of the mucosa. As a result, there may be an accumulation of absorbed drug in the mucosal cells. The decrease in blood flow may, in effect, diminish the concentration gradient across the intestinal mucosa, which is required for optimal absorption of drugs transported into the blood by a passive diffusion process.

Variations in intestinal blood flow can alter the rate but are unlikely to affect the extent of availability, unless the drug is subjected to extensive intestinal metabolism. An interesting clinical example is provided by Rowland et al (1972) who observed that aspirin absorption ceased promptly when a subject fainted while blood was being withdrawn from a peripheral vein and commenced again as the subject recovered. However, the extent of availability was the same as on another occasion when the volunteer did not faint and absorption of aspirin was rapid.

One more point that should be made is that any potential enhancement of drug absorption rate by increase in the blood flow is offset by concomitant slowing of gastric emptying (Brandt et al,1955) or binding to or interacting with components of food (McLean et al, 1978).

(c) <u>The lymphatic route</u>

Although the lymphatic route is rarely considered where drug absorption is concerned, the fact is that any substance, which is transferred through the intestinal epithelium into the lamina propria, has equal access to both blood and lymph capillaries (De Marco and Levine, 1969). Also, material which leaves the intestine via lymph gains access to the general blood circulation without first passing

through the liver, thereby averting potential metabolism prior to distribution.

Ordinarily, a much greater proportion of a small molecular species is absorbed via the blood than via the lymph because the rate of blood flow is several hundred times that of lymph flow (De Marco and Levine, 1969). For example,these authors have shown that only a small percentage of a well absorbed drug, p-amino-salicylic acid, or an inefficiently absorbed drug, tetracycline, was absorbed via lymph under normal conditions. However, the amount of these agents carried away in the lymph was doubled when lymph flow was stimulated by administration of tripalmitin. While this increase in the amount of absorption via lymph would have little therapeutic consequence in the case of the two drugs just mentioned, such an increase might have relevance to the effectiveness of oral therapy with agents which are only slightly absorbed at best and which are susceptible to hepatic metabolism. In this respect it is well to remember that the lymphatic route is the major pathway for absorption of large molecules, such as cholesterol, protein and fatty acids.

Anticancer agents have been reported to be removed to a great extent from the site of administration via the lymph (Takahashi et al, 1973; Nakamoto et al, 1975a and b; Hashida et al, 1977a and b) when administered as emulsion dosage forms, particularly water in oil emulsions. In fact, even with Mitomycin C and bleomycin, which have relatively low molecular weights, enhancement of these agents was reported and even without discernible change in lymph flow (Nakamoto et al, 1975a and b). The existence of a special transport mechanism by which drug and oil were delivered together when injected

intramuscularly, intraperitonealy or at the stomach wall was proposed. These agents could be transported from the site of administration directly into the lymphatic system, either with the oil droplet carriers or as free drug after separation at the injection site. The significant contribution of the former route was suggested from the parallel increased transport of the drug and tripalmitin following injection of emulsions. Fig. 1.1 is taken from Hashida et al (1977b) to show these possibilities in the case of iodohippuric acid.

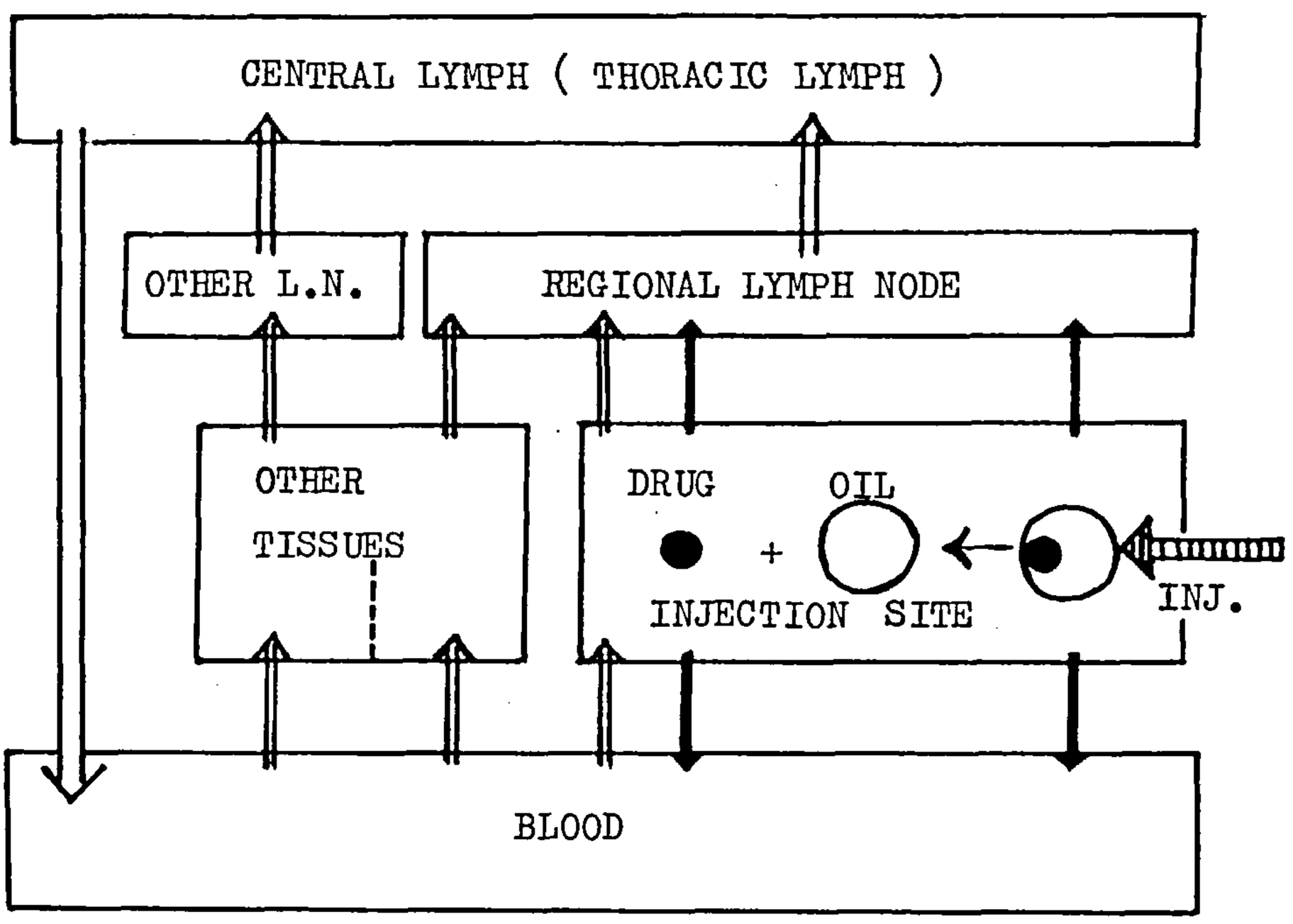

Fig.1.1 Model of lymphatic transfer of iodohippuric acid following the injection of emulsion formulations.

(d) Intestinal metabolism

Drugs can undergo metabolic transformation at various sites along the GI tract. Drug metabolizing enzymes are present within the GI fluids, the microflora (Scheline, 1968), and the intestinal epithelial cells (Barr and Riegelman, 1970a). The importance of the metabolic alterations of a drug within the tract in terms of bioavailability will depend upon the rate and extent of metabolism, the absorbability of the metabolite, and its pharmacological activity relative to the parent drug.

Intestinal fluids of the dog and specific components of those fluids (e.g. pancreatin, trypsin, etc) and the intestinal mucosa of the rat are capable of deacetylating N-acetylated drugs, e.g. N-acetylsulphisoxazole (Randall et al, 1954). Various drugs containing ester groups may be hydrolysed by specific or non specific esterase enzymes present in the GI fluids. Chloramphenicol palmitate is hydrolysed by contents of the rat duodenum and by purified lipase enzymes (Glazko et al, 1952). The acetoxymethyl ester of benzyl penicillin is de-esterified prior to reaching the portal circulation in dogs, suggesting breakdown by esterase enzymes in the gut fluids or within the intestinal membrane (Agersborg et al, 1966).

The metabolic potential of the GI microflora has been recognised only recently, In normal subjects, the stomach and proximal small intestine contain relatively small numbers of microorganisms, while larger numbers are seen toward the distal end of the small intestine. These microflora, which arise primarily from the environment, tend to adhere to the luminal surface of the intestine. Within an individual, the microflora population and type appear to

remain rather stable over long periods of time (Gorbach et al, 1967). The primary factors (Donaldson, 1973) governing the number and kind of micro-organisms present in the tract are (i) gastric secretions, which limits the growth of these organisms in the stomach and upper regions of the tract, and (ii) the propulsive motility of the intestine, which is responsible for continually cleansing the tract and thereby limiting the proliferation of these organisms. Gastric atrophy permits increased numbers of micro-organisms to pass into the small intestine. Similarly, reduced intestinal motility results in overgrowth of these flora.

There have been several reviews of drug metabolism by intestinal micro-organisms (Scheline, 1968 and 1973; Smith, 1971; Goldman et al, 1974). The majority of studies have been done in laboratory animals rather than man so that the clinical implications of drug metabolism by the microflora are difficult to assess.

In addition to drugs being metabolised by the GI enzymes and gut flora, there are several examples of metabolism within the cells of the intestinal membrane. Barr and Riegelman (1970b) have shown that salicylamide is glucuronidated by the rabbit intestine and it is suggested that this may occur in man (Barr, 1969). A recent study in dogs has indicated that approximately 42% of an oral dose of salicylamide is metabolised within the intestinal wall (Gugler et al, 1975). A significant decarboxylation of L-dopa during passage through the gut wall was reported(Mearrick et al, 1975).

A variety of factors affect the drug metabolism. As the metabolic processes have limited capacities (Mearrick et al, 1974 and 1975; Wade, 1980), gastric emptying rate, therefore, plays a significant role in the case of drugs subject to pre-systemic

metabolism (i.e. first pass effect and/or intestinal metabolism). Mearrick et al (1974) attributed the increase in bioavailability of L-dopa when co-administered with metoclopramide to the more rapid delivery to the site of absorption. As the gut metabolism has a more limited capacity than the transport mechanism, there is an increase in the availability when gastric emptying is stimulated. Furthermore, the time to obtain peak plasma concentrations is reduced and multiple peaks in the absorptive profile are eliminated. Delay in GER has the opposite effect since this increases the exposure of the drug to the destruction by enzymes (Wade et al, 1974; Mearrick et al, 1974).

In addition, slow transit rate through the GI tract, by, for example, reduction or inhibition of propulsive movement, would increase the metabolism of the drugs that are metabolised by microflora. This is not only because of increase in the number of these microflora but also because the drug may reach the site of metabolism in a form available for metabolism.

Among other factors affecting drug metabolism are:- age, sex, diet, alcohol and other drugs. The most profound age-related differences occur between the adult and the neonate. Neonates appear to have a low drug-metabolism capacity compared to adults. This deficiency is responsible for the serious adverse effects observed after administration of chloramphenicol (Weiss et al, 1960). A markedly prolonged half-life of tolbutamide has been reported in neonates compared with adults (Nitowsky et al, 1966).

Drug metabolism within the GI tract, at any of the several sites discussed above, offers a plausible explanation for the poor bio-availability of certain drugs.

CHAPTER 2

THE REGULATION OF GASTRIC EMPTYING RATE BY FATS AND BY OSMOTIC PRESSURE

As will be seen from chapter 4 in this section the work described in this thesis is concerned with the effects of certain oily suspension vehicles on the bioavailabilities of drugs. In addition to oil some of these vehicles contain large proportionsof sugar that are added to improve the suspension properties of the vehicles. Since it has already been indicated that oils and sugars affect GER (see 1.4.1a in previous chapter) the mechanisms of these effects are considered in more detail in this chapter.

Shay and Gershon-Cohen (1934) studied the effect of a number of solutes on the GER and concluded that the acceptability of the gastric contents to the duodenal mucosa provides the primary control of gastric emptying. The arrival of the initial portion of a meal in the duodenum serves as a test of the acceptability of the gastric contents. Thus, the use of the Lintvarev term 'trial portion', as described by Shay and Gershon-Cohen (1934), is appropriate. If acceptable, this trial portion advances and further gastric emptying follows. However, should this portion be unacceptable, stimulation of the duodenal mucosa occurs and a reflex is activated, which results in pyloric closure. Closure is maintained until the trial portion is sufficiently neutralized or diluted to permit its passage to the adjacent distal duodenum. Pyloric relaxation then takes place and further gastric emptying follows. Shay and Gershon-Cohen (1934) stressed their belief that the pyloric state, rather than peristaltic movement or gastric tonus, is the most important

factor in gastric emptying. Furthermore, they indicated that induction of pylorospasm is rapidly followed by a cessation of gastric peristalsis, and a return of active peristalsis is not usually seen until the agent producing pylorospasm has been removed. This latter point has been confirmed by more recent work (Quigley et al, 1941; Quigley and Meschan, 1941; Brink et al, 1965; Fisher and Cohen, 1973) as will be discussed in part 2.2 of this chapter.

A receptor theory for the regulation of GER has been proposed (Hunt, 1956; and 1963; Hunt and Knox, 1968a and b; Bell et al, 1972; Cooke and Christensen, 1973; Cooke, 1975; Hunt, 1975). The receptors, which slow GER in response to the composition of the duodenal contents, are located on the wall of the duodenum. There are no grounds for supposing that there are duodenal receptors which accelerate gastric emptying. Thus, a maximal rate of gastric emptying is assumed to correspond to minimal stimulation of duodenal receptors, which inhibit peristalsis and raise pyloric pressure. According to the above authors there are three types of receptors, i.e. fat, acid and osmoreceptors. The remaining discussion is limited to a consideration of the first and last of these three types.

2.1 Osmotic pressure and gastric emptying rate

On general grounds it would be expected that solutions iso-osmotic with plasma would be least disturbing to a tissue. In addition, it is customary to think of the stomach as a protective reservoir for the intestine.

The idea that the osmotic pressure of gastric contents might influence GER stems from the observations of Carnot and Chassevant

(1905). They found that, in dogs with a duodenal fistula, saline of the same osmotic pressure as plasma left the stomach more rapidly than more dilute or more concentrated solutions. Isotonic solutions produced a series of pyloric openings, which permitted the solutions to pass very rapidly by successive jets from the stomach to the intestine without undergoing great modifications. If, however, the solutions were appreciably non-isotonic contact of the first portion of the liquid with the duodenum provoked reflex closure of the pylorus, which did not reopen until proper dilution of this portion had taken place. Since that time there has been confirmatory work by Appearly (1926), Johnston and Ravdin (1935), Shay (1944), Wells and Welbourn (1951)and Jones (1951).

Hunt (1954 and 1956) studied the effect on gastric emptying of a selected range of solutes in test meals. In these experiments the concentration of the solutes in the meal was varied and recoveries were made at a constant time after giving the meal by tube. For example, the volume of the gastric contents was greater at 30 min (Hunt, 1954) and 20 min (Hunt, 1956) with meals containing 35 gm sucrose/dm^3 than it was with test meals containing no sucrose. Additional experiments (Hunt and Pathak, 1960) showed that different solutes were all approximately equivalent per osmole in slowing gastric emptying, thus suggesting that a single receptor was responding to some common property. The finding that many solutes have an equal action per osmole makes it difficult to dismiss the idea that there is an osmoreceptor which slows gastric emptying (Hunt, 1961 and 1963).

The mechanism by which this osmoreceptor regulates GER stems from the observations of Hunt (1961 and 1963) and has been reviewed by Bell et al (1972) and Cook and Christensen (1973).

It is suggested that the receptor is in the form of a vesicle having some of the properties of a red blood cell, i.e. it is deflated by contact with hypertonic solutions. Such deflation is postulated to cause a signal to be relayed to the stomach where it inhibits the peristaltic action of the GI pump. This would allow the duodenal secretion to dilute the gastric efflux to a greater extent and thus reduce the stimulus to the osmoreceptor. On the other hand, inflation of this osmoreceptor with substances that penetrate it along with water from the duodenum is postulated to reduce the signal that inhibits gastric emptying. Glucose, sucrose and sorbitol are believed not to penetrate into the osmoreceptors. Thus, deflation of the osmoreceptors gives slow emptying and inflation gives rapid emptying of the stomach.

The site of the osmoreceptor has been reported to be in the duodenum and jejunum (Hunt, 1961, 1963 and 1975; Hunt and Knox, 1968a and b; Bell et al, 1972; Cook and Christensen, 1973; Cook, 1975). Evidence for this supposition arises from the fact that isocaloric meals of starch and glucose empty at the same rate and more slowly than water. Since hydrolysis of starch occurs beyond the pylorus, the osmoreceptor must be there also.

This mechanism explains and supports the earlier theory and observations of Carnot and Chassevant (1905), Appearly (1926), and Shay and Gershon-Cohen (1934). Cook and Christensen (1973) believe that this osmoreceptor mechanism could be mediated by a humoral effect.

2.2 Fats and Gastric emptying rate

It has been known since the first observations of Edwald and Boas (1886) that fat introduced into the stomach inhibits its secretions and motility. This inhibitory effect was demonstrated by Lintvarev (1901), Ivy and Farrel (1925), Farrel and Ivy (1926), Kosaka and Lim (1930), Roberts (1931), Waugh (1936), Card (1941), Tidwell and Cameron (1942) and Grossman (1950), who recognised that inhibition takes place mainly from the duodenum and is a function of the chemical nature of the fat rather than its physical properties. The effects of many neutral triglycerides on GER were tested and it was concluded that unsaturated fats were the more effective inhibitors. The effect was only observed (Roberts, 1931; Quigley et al, 1934; Waugh, 1936) after the fat had left the stomach. It was concluded that either oil absorption exerts this inhibitory effect on gastric motility or some substance, which acts in a similar manner, is formed or liberated. This inhibitory substance may be formed in the mucosa (Kosaka and Lim, 1930) of both the small and large intestine as a result of contact with fat. This provides further evidence to substantiate a humoral theory of inhibition. The name enterogastrone was suggested for the intermediary substance (Kosaka and Lim, 1930; Grossman, 1950).

The presence of fat in the intestine does not inhibit gastric motility or secretion unless both bile salts and lipase are present in the lumen of the intestine. This conclusion is based on the following facts. Introduction of 1 cm^3 of fat into the proximal small intestine of intact rats consistently suppressed gastric motility. In rats with diversion of bile from the small intestine this inhibition failed to occur unless bile salts were added to the

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administered fat. When both bile and pancreatic juice were diverted from the small intestine, inhibition occurred only when oil was mixed with bile salts and lipase prior to injection (Menguy, 1960). Gastric emptying of fatty meals is significantly more rapid in patients with pancreatic insufficiency compared with normal subjects. Addition of pancreatic enzyme to fatty meals slowed the gastric emptying in these patients. Pancreatic enzyme had no effect when added to 5% glucose solution or a fatty meal of normal subjects. These results suggested that the digestive products of fats inhibit GER (Long and Weiss, 1974). Bile salts administered parenterally or intraperitoneally inhibited gastric secretion and motility in rats and dogs (Menguy, 1959a and b; Menguy and Koger, 1959; Menguy and Peisner, 1960). Since bile salt secretion is enhanced (see part 1.4. 2b in the previous chapter) during fat absorption, it was concluded that the reabsorbed bile salts could be the humoral agent mediating gastric inhibition by fat. The specific inhibitory effect of lipolytic products per se was ruled out by Quigley et al (1934).

More recently it has been reported that the sodium salts of fatty acids (Hunt and Knox, 1968c) and digestive products (Hunt, 1975) caused the inhibition in GER, since monoglyceride scarcely slows gastric emptying. Hunt (1975) showed that fatty acids in the form of soaps gave a greater reduction in GER and thus supported the finding of Quigley and Meschan(1941), who found that the order of retardation of gastric evacuation, as a result of pyloric closure and inhibition of antral activity, was soap > fatty acids > neutral fats.

The idea that delay in GER is caused by the digestive products of fat has been supported recently. Inhibition occurs within 5 minutes

(Moberg and Carlberger, 1974), which is exactly the same time as reported by Farrel and Ivy (1926). The pattern of emptying was described by Moberg and Carlberger (1974) as composed of a rapid initial phase (5 minutes) followed by a prolonged emptying phase, during which a remarkable inhibition of gastric emptying was noted with fat containing meals. These findings and others (Moberg et al, 1974) suggested that the emptying rate during the prolonged phase was evoked in the upper small intestine by the digestion and absorption of fat. Knox and Mallinson (1971) demonstrated that triglycerides must be digested to exert their inhibitory action. A relationship has been reported between the GER and digestibility and absorbability of the fat (Johansson et al, 1972; Yamahira et al, 1978). The latter conducted further studies and found that increase in the volume of oil caused more delay in GER.

Having these points in mind, together with the fact that earlier studies (Roberts, 1931; Quigley et al, 1934; Waugh, 1936) showed that inhibition in GER only occurs when fat leaves the stomach and passes through the pyloric sphincter, it can be concluded that the fat receptor is located somewhere beyond the pylorus, and that it is sensitive to the digestive products of fat.

Fatty acids are known to decrease GER via sensitive receptors located in the duodenum and jejunum (Shay et al, 1939; Hunt and Knox, 1968a and b; Cook and Christensen, 1973; Hunt, 1975; Cook, 1975). The resulting inhibition of GER was found to be correlated to a significant inhibition of the propulsive movement in the duodenum by sodium myristate (Borgstrom and Arborelius, 1975). Once the gastric contents had filled the duodenal bulb, no further emptying occurred

before the bulb had been partially or completely emptied by an antegrade (i.e. propulsive) peristalsis wave. The authors thought, therefore, that duodenal transport rate interferes directly with the GER. Hunt and Knox (1968b) and Weisbrodt et al (1969) also believed that the duodenal contractility and motility regulate GER, but in a different way. They thought that the antral contractility and duodenal quiescence are intermittent during gastric emptying. Weisbrodt et al (1969) found that the slowest rate of emptying occurs when there is a relatively low antral and a high duodenal activity and vice versa.

However, it seems that the conflicting ideas of these workers can be explained as follows. The emptying of material that is incompatible with the duodenal mucosa into the duodenal bulb leads to enhanced duodenal activity since the normal antegrade (propulsive) waves are followed by retrograde peristaltic waves that prevent emptying of the duodenal bulb. This leads, in turn, to inhibition of gastric emptying, i.e. antral activity decreases. Thus, the resultant delay in transport from the stomach to duodenum allows the duodenum time to handle the initially incompatible material. When compatibility has been ensured duodenal activity decreases and gastric emptying occurs because antral activity then increases, i.e. there are intermittent periods of antral and duodenal activity.

However, the above mechanism does not take into account the fact that the pyloric sphincter may also interfere with gastric emptying. Waugh (1936) and Gershon-Cohen and Shay (1937)showed, using radiological observations,that fat in the duodenum and jejunum immediately produced marked atony of the whole stomach, with no

peristalsis for 30-90 min, together with pyloric closure. In addition, increase in the pressure exerted by the pylorus has been reported when olive oil is instilled into the duodenum in the dog (Brink et al, 1965) but only after 15-20 min, whereas antral motility was inhibited immediately. The role of the pyloric sphincter and inhibition of the peristaltic movement of the stomach in regulating GER have been reported in response to fat in the duodenum of the dog (Quigley and Meschan, 1941; Quigley et al, 1941) and to olive oil and glucose in the duodenum of man (Fisher and Cohen, 1973).

Fisher and Cohen (1973) strongly suggested that the endogenous release of cholecystokinin and secretin augment the pyloric pressure. Their suggestion, which was supported by the work of Fisher et al (1973),stems from the facts that secretin inhibits gastric emptying and secretion in man (Chey et al, 1970); cholecystokinin, the production of which is stimulated by fat, (Isenberg and Csendes, 1972), delays gastric emptying in man (Chey et al, 1970) and raises pyloric pressure in dog (Isenberg and Csendes, 1972); and that olive oil raises the pyloric pressure after being introduced into the duodenum (Brink et al, 1965). However, enterogastrone, the postulated hormone, has not yet been isolated in pure form. It refers to a substance, liberated from the duodenum and jejunum by fats, which inhibits both gastric emptying and secretion. It is neither cholecystokinin nor secretin (Johnson and Grossman, 1969). Its structure has been partly determined; it appears to be distinct from other GI hormones (Brown et al,1970) and does not possess any significant cholecystokinin or secretin activity (Brown et al, 1969).

In summary, GER has been suggested to be regulated by receptors that are located in the duodenum and jejunum and that are sensitive to osmotic pressure and the digestive products of triglycerides, namely fatty acids. As a result of stimulation, enterogastrone would be released and this mediates the delay in GER through myogenic activity of the GI tract, i.e. the pyloric pressure region is raised and the GI peristaltic motility is altered.

The relative effectiveness of this inhibition has been reported. For example, the following test meals would decrease the GER to the same extents; 500 millimoles of glucose and 8 mmole myristic acid per litre (Hunt and Knox, 1968b; Cook, 1975); 9 gm carbohydrate/100 cm^3 and 4 gm triglycerides/100 cm^3, both equal to 36 kcal /100 cm^3 (Hunt, 1975; Hunt and Stubbs, 1975).

CHAPTER 3

THE ENHANCEMENT OF INTESTINAL ABSORPTION OF DRUGS BY FATS AND OILS

In addition to the major effect of oils in delaying GER, which was the subject of the previous chapter, the formation of bile salt-fatty acid-monoglyceride mixed micelles, that are formed during the digestion of fats and oils (Senior, 1964; Dawson, 1967; Hofmann and Small, 1967; Carey and Small, 1970; Holt, 1972; Ockner and Isselbacher, 1974), has been reported recently to have a significant effect on the enhancement of intestinal absorption of drugs which are normally, poorly absorbable, e.g. streptomycin and gentamycin or nonabsorbable, e.g. heparin. The following mechanisms have been proposed to explain this effect; (a) direct interaction of the drug with the mixed micelles, (b) the enhancement in the absorption is related to the digestibility and absorbability of the lipids, (c) alteration of the mucosal membrane permeability.

Based on in situ absorption studies in the rat, using intestinal loops closed at proximal and distal ends, Inui et al (1976) and Tokunaga et al (1978) suggested that the mixed micellar solution enhanced drug absorption by mechanisms (a) and (c). Using the same method of study Muranishi et al (1977 and 1979) and Muranushi et al (1980a) suggested that the enhancement of absorption was unlikely to be due to (a) and (b). The alteration of the mucosal permeability was examined by exorption studies using sulphanilic acid, and by pretreatment of the intestinal loop with buffer solutions as controls, with micellar solutions of bile salts and mixed micellar solutions. A close correlation was found between the enhancing effect and the alteration of the mucosal membrane permeability. It was concluded that the enhancement of intestinal absorption of drugs by the addition of

mixed micelles is mostly due to an increased permeability of the mucosal membrane caused by the incorporation of the lipid component of the mixed micelle (Muranushi et al, 1980a).

Since the mixed micellar solution significantly enhanced the absorption over that of a bile salt micellar solution alone (Muranishi et al, 1977 and 1979; Muranushi et al, 1980a), it was thought that the lipid of the mixed micelle played a critical role in this enhancement (Taniguchi et al, 1980; Muranushi et al, 1980a and b). This led Taniguchi et al (1980) to conduct further experiments using different lipids and different surfactants, including bile salts, to study the effect of surfactant mixed micelles. Although bile salt micellar solution did not cause a marked increase, an addition of mono-olein or oleic acid to that solution resulted in an induction of the absorption. Mono-olein and oleic acid caused a remarkable effect irrespective of the surfactant present. Another interesting finding by the same authors was the ineffectiveness of triglycerides, such as triolein and trioctanoin, so the great efficacy of absorption of oleic acid plays an important role in the improvement of heparin absorption.

Furthermore, polar lipids, administered as adjuvants, can also be absorbed from the intestine, penetrating through the epithelial cells. The disappearance of mono-olein or oleic acid from the intestinal lumen was remarkably rapid and occurred within 15 min. This disappearance appears to be highly correlated with the absorption rate of heparin. In addition, the absorption of heparin was higher from mono-olein-bile salt solution than from mono-olein-HCO 60 solution (HCO 60 is a non-ionic surfactant, i.e. the polyoxyethylene derivative of hydrogenated castor oil), and concomitantly mono-olein

itself was absorbed in higher amounts from the bile salts solution than from the HCO 60 solution (Taniguchi et al, 1980). The authors suggested that some relationship exists between the penetration of polar lipids into the epithelial cell membrane and the delivery of heparin molecules into the cell. It follows, therefore, that polarity and digestibility of the lipid play a critical role in the absorption of the drugs (Bloedow and Hayton, 1976; Yamahira et al, 1979).

Further studies were conducted by Muranushi et al (1980b) on the mechanism of the intestinal absorption of drugs in the presence of mixed micelles using the liposomal membrane as a biomembrane model. The effect of incorporating various lipids on the permeability of drugs through the liposomal membrane was investigated. A close correlation was observed between the enhancement of intestinal absorption of drugs induced by the mixed micelles and the altering of the permeability of the liposomal membranes by the incorporation of the lipid component of the mixed micelles. Namely, lipids which enhanced the intestinal absorption increased the permeability of liposomal membranes, and lipids, which did not cause an increase of the drug absorption, did not alter the permeability of liposomal membrances. Furthermore, the degree of the enhancing effect of various fatty acids on the permeability of liposomal membranes corresponds to the extent of the enhanced intestinal absorption in the presence of fatty acids mixed micelles.

The role of bile salts, however, should not be neglected particularly with regard to their contribution in altering membrane permeability and their involvement in fat absorption. The principal

role of the bile in facilitating lipid absorption is to solubilise the products of lipid digestion. Fatty acids then exist as monomers in true solution in equilibrium with the fatty acid within the mixed micelles. The actual process of fatty acid absorption occurs through the monomer phase (Westergaard and Dietschy, 1976).

Based on these facts, the mechanism for the inducement of intestinal absorption of poorly absorbed drugs by mixed micelles is speculated by Muranushi et al (1980b) as follows: " The micellar state may facilitate the incorporation of the lipid component of mixed micelles into the mucosal membrane. The incorporated lipid interacts with the polar region of the membrane phospholipids and enhances the fluidity and permeability of the mucosal membrane. Consequently, poorly absorbed drugs can transfer across the mucosal membrane easily."

Finally it should be mentioned that all the above studies were conducted using closed intestinal loops. Therefore, the major effect of the fat in delaying gastric emptying rate was not taken into account.

CHAPTER 4

SCOPE OF THE THESIS

Suspension dosage forms are important classes of pharmaceutical products. These dispersion systems present many formulation, stability, manufacturing and packing challenges.

Oral pharmaceutical suspensions have been known and used for a long time. Besides being the dosage form that is favoured by the very young and by elderly people, who find it difficult to swallow tablets or capsules, several other reasons exist for the use of suspensions (Ansel, 1976).

Oral pharmaceutical suspensions are comprised of a physiologically active ingredient(s) and the vehicle. The vehicle is generally comprised of a liquid plus density and viscosity enhancing agents, flavouring agents and preservatives. Water has usually been the preferred liquid. The physiologically active agent is present as dispersed particles, the size of which is usually very small. The solubility of the active agent in the liquid is very low.

The oral pharmaceutical suspension is contrasted with the oral pharmaceutical solution in which the physiologically active agent is dissolved in the liquid. Where certain drugs are chemically unstable when in solution the oral suspension improves chemical stability whilst permitting liquid therapy (Ansel, 1976).

In many cases, however, the physiologically active agent is not chemically stable in either a water-based oral pharmaceutical suspension or an oral pharmaceutical solution. In such cases it is impossible to prepare a satisfactory liquid pharmaceutical dosage form utilising water as the base for the preparation. Even when the drugs

are supplied as powders to which water can be added immediately before use, drug degradation may still occur during daily use by patients. Consequently, the use of a non-aqueous vehicle, e.g. an oily vehicle, would seem reasonable in these cases.

Although an oily vehicle is not well accepted because of its taste, Stephens and Su (1975) claim the following advantages; (a) it is useful for preparing oral pharmaceutical liquid dosage forms of water degradable physiologically active ingredients, (b) it has good flow properties, and (c) it shows resistance to the settling and caking of suspended particles. In addition, oily vehicles may enhance the GI absorption of drugs, as indicated later in this chapter.

Some oily vehicles for pharmaceutical formulations have been the subject of various patents. For example, an oily vehicle patented by Stephens and Su (1975) is composed of refined fractionated coconut oil containing hydrogenated castor oil, lecithin, aluminium stearate and an oil insoluble excipient, such as sucrose, to adjust the viscosity of the vehicle. Preservatives and flavouring agents can be added to modify the oily taste. Another oily vehicle discovered by Lin and Pramoda (1978) is composed of sesame oil, silica gel (Cab-o-sil) and sucrose. However, these patents were concerned mainly with the physical and chemical stabilities of given suspensions rather than with their bioavailability aspects. Although Lin and Pramoda (1978) conducted simple crossover studies to compare the bioavailability of a permanent suspension of amoxicillin in their novel oily vehicle with that in the commercially available aqueous suspension, their studies were limited. However, extensive stability tests confirmed that the novel oily suspensions exhibited excellent

stability, with shelflives exceeding five years in some cases (as, for example, with ampicillin) with minimal oil separation. Similar stabilities were reported by Stephens and Su (1975) in the assessment of their vehicle, together with claims that oily taste was not apparent and that the vehicle has a good 'mouth feel'.

Little information is available on the factors that may affect the bioavailability of drugs administered orally as a suspension, particularly in oily vehicles. Although it is well known that fat enhances the bioavailability of many drugs most studies have been conducted by coadministration of fatty meals with drugs in man (Crounse, 1961 and 1963; Kabasakalian et al, 1970; Bates et al, 1974b; Rosenberg and Bates, 1976; Melander et al, 1977a,b and c; Melander and Wahlin, 1978; Koch et al, 1978; Melander et al, 1979). Other studies have demonstrated the enhancement of bioavailability of drugs from emulsion dosage forms in man (Wagner et al, 1966; Bates and Sequeira, 1975; Bates et al, 1977) and in the rat (Kaiser et al, 1967; Carrigan and Bates, 1973; Chakrabarti and Belpaire, 1978; Ogata and Fung, 1980). However, the GI absorption of drugs administered orally to intact animals in oily suspension dosage forms has received very limited attention, e.g. in mice (Feinstone et al, 1940), and in rat (Carrigan and Bates, 1973; Bloedow and Hayton, 1976; Chakrabarti and Belpaire, 1978). No studies have explored the various factors that affect the drug release and absorption from oily suspension dosage forms, apart from that of Bloedow and Hayton (1976). Even this study was limited to the use of a simple dispersion of the drug in the oil, i.e. no attempts were made to consider the effects of other pharmaceutical adjuvants, which are necessary in

the production of commercial oily suspension dosage forms.

In view of the above comments the work presented in this thesis was carried out in order to investigate the effects of oil on the bioavailability of sodium salicylate, ampicillin trihydrate and nitrofurantoin in either rabbits or rats. In addition, the effects of the different pharmaceutical adjuvants and different concentrations of the suspending agents, that are specified in the two patents mentioned above, were investigated, individually and in combination, on the bioavailabilities of the three drugs.

With the factors that influence the dissolution and absorption of drugs in mind (see parts 1.3 and 1.4 of Chapter 1 in this Section) some of the physicochemical properties of the drugs have been studied, e.g. solubility of the drug in the oil and in 0.1 mole/dm^3 HCl, apparent partition coefficient between the oil and the acid. Rheological properties of dispersions of each ingredient and combinations of all the ingredients specified in the above mentioned patents, together with different concentrations of the suspending agents were also studied. The possibility of adsorption of the drugs from solution in the oil onto the insoluble suspending agent (Cab-o-sil) was studied in an attempt to explain the effect of this suspending agent on the bioavailabilities of the drugs. Finally, in order to investigate the extent of the effect of viscosity on the results obtained using the oily vehicle in vivo and the possibility of correlating the in vivo results with in vitro measurements two classical in vitro dissolution methods, i.e. flask-stirrer and dialysis methods, were used to follow the release of the drugs from the suspensions.

SECTION 2

RHEOLOGICAL STUDIES ON OILY VEHICLES

CHAPTER 1

RHEOLOGICAL STUDIES ON OILY VEHICLES

1.1 Introduction

The rheological properties of a pharmaceutical dosage form, which can range in consistency from liquid through semisolid to solid, can affect its patient acceptability, physical stability, and the biological availability of the active ingredient.

With respect to the physical stability of suspension dosage forms, i.e. the ability of the suspension medium to retain insoluble particles in a suspended or substantially suspended easily redispersed state, the viscosity of the suspension vehicle is an important factor as indicated by Stokes' law, which is described by Eq. 1.1

$$v = \frac{d^2(\rho_1 - \rho_2)g}{18\eta} \qquad \text{Eq. 1.1}$$

where v = the sedimentation rate, g = gravitational constant, d = the diameter of the particle, ρ_1 and ρ_2 are the densities of the particle and dispersion medium, respectively and η is the viscosity of the dispersion medium.

According to Stokes' law, an increase in the mean particle size or in the differences between the densities of the solid and liquid phases will produce a faster rate of sedimentation, while an increase in the viscosity of the liquid medium will decrease the sedimentation rate (Richards, 1972).

In addition to viscosity, other rheological properties of the suspension medium, such as thixotropy and yield value can be related

to suspension stability. For example, Foernzler et al (1960) found a direct relationship between the sedimentation rate and the reciprocal of the thixotropic area of zinc oxide suspensions and Meyer and Cohen (1959) reported suspensions of some drugs suspended in a plastic medium to be permanent when the medium exhibited a critical minimum yield value, irrespective of the apparent viscosity.

These rheological properties, i.e. thixotropy and yield value, have been used to promote prolonged drug action *in vivo*. For example, Ober et al (1958) reported that concentrated (40-70% w/v) aqueous procaine penicillin G suspensions are highly thixotropic and possess exaggerated yield values. This yield value was the key to the sustained action of the injectable products of Ober et al. Suspensions possessing high yield value were very thixotropic, and the authors found that these suspensions first became fluid as they passed through the hypodermic needle, then quickly recovered their structure to form a complete depot in the muscle, thereby providing prolonged therapeutic blood levels.

The rate of structural recovery was found by Ober et al to be important and, in fact, is of significance in all suspensions where thixotropy is employed to achieve stability. This is the case with oily thixotropic aluminium stearate gels (Buckwalter and Dickison, 1948 and 1958) as will be discussed later in this introduction. Therefore, rheology is of a great importance with respect to dissolution and release *in vitro* as well as drug release and bioavailability *in vivo*.

Whilst the rheological aspects of aqueous suspension media have received considerable attention with respect to drug release and dissolution *in vitro* (Kabre et al, 1964; Braun and Parrott, 1972; Florence et al, 1973; Bachynsky et al, 1976; Shah and Sheath, 1976;

Barzegar-Jalali and Richards, 1979a) and drug release and bio-availability *in vivo* (Levy and Jusko, 1965; Hewitt and Levy, 1971; Levy and Roa, 1972; Barzegar-Jalali and Richards, 1979b; Marvola et al, 1979a; Soci and Parrott, 1980), little attention has been paid to these aspects in the case of non-aqueous vehicles. In addition, the small number of publications concerned with the effects of the rheological properties of such vehicles have been limited to dermatological products, e.g. Kostenbauder and Martin (1954), Billups and Sager (1964); Whitworth and Stephensons (1971), Asker and Whitworth (1974) and Davis and Khanderia (1980), or to injectables, e.g. Buckwalter and Dickison (1948 and 1958) and Phadke (1975). Thus, there is no apparently available information concerning the effects of the rheological properties of non-aqueous vehicles on the bio-availabilities of orally administered drugs. In fact, there is even little information on the rheological properties of such vehicles.

Aluminium stearates have been used as a suspending agent and gelling agent in oily vehicles intended for intramuscularly injected repository forms of penicillin by Buckwalter and Dickison (1948 and 1958). Appropriate rheological properties were included in the list of requirements suggested by these workers, e.g. the injection should (a) be capable of easy withdrawal into a syringe and administration at room temperature, (b) retard the release of drug from the site of injection and (c) prevent the sedimentation of suspended drug particles. Buckwalter and Dickison (1948 and 1958) indicated that the rheological properties of the oily gels depend on the type of aluminium stearate that is used (i.e. mono, di- or tristearate), on the nature of the oil and on the conditions under which the gel is prepared. They concluded that gelled vehicles that satisfied their

list of requirements were obtainable. Later work by Phadke (1975) showed that the sedimentation rate of procaine benzylpenicillin in sesame oil gelled with aluminium stearate correlated well with the duration of penicillin blood levels in the rabbit. In fact, this worker suggested that sedimentation rate would provide a reasonable index of batch quality and might provide a substitute for blood level measurements.

Aluminium stearate (50:50 mixture of mono- and distearates) is also an essential ingredient in the oily vehicle patented by Stephens and Su (1975) for use as a suspension medium for orally administrated drugs that are water degradable, such as erythromycin, penicillin-v, tetracycline, cephalexin and others. The patent claims that the drug suspensions possess good physical stabilities and infers that the vehicle exhibits appropriate rheological properties. However, direct assessment of these properties is not described.

Colloidal silica has also been used as a thickening agent for non-aqueous vehicles and Lin and Pramoda (1978) have patented such a vehicle for use with orally administered drugs that are not stable in aqueous suspensions, e.g. amoxicillin and ampicillin. Zia et al (1974) also showed that the stability of penicillin G in peanut oil thickened with silica could be improved by reducing the surface acidity of the silica. The effect of this suspending or gelling agent on the in vitro release of methylsalicylate from n-dodecane and 1-dodecanol has been studied by Sherriff and Enever (1979).

In spite of the importance of the rheological properties of vehicles gelled with either aluminium stearate or colloidal silica these properties were not specified for the oils and gels used in the above studies except for the systems investigated by Sherriff

and Enever (1979). Previous work on the flow properties of aluminium soap-hydrocarbon systems has been reported (Shiba, 1960; Stephens, 1971) but these studies were limited to systems containing liquid paraffin and many aspects of the rheological behaviours of these systems were not fully explained.

In contrast to most of the previous studies the flow curves of the oily vehicles used in the present work are described in this chapter.

1.2 Experimental

1.2.1 Materials

Fractionated Coconut Oil (FCO) B.P.C. 1968, was obtained from Alembic Products Ltd. The acid and saponification values of the oil were checked every two weeks during the course of the whole investigation to ensure that they remained within the limits described in the B.P. (1973). Colloidal silica (Cab-o-sil) and lecithin 90% (refined grade) were obtained from B.D.H. Chemicals Ltd. Aluminium mono-and distearates were obtained from Witco Chemical Ltd. Hydrogenated castor oil was obtained from Akzo Chemie U.K. Ltd. Sucrose (Icing sugar) was obtained from the British Sugar Corporation Ltd. and xanthan gum (Keltrol-food grade) was obtained from Kelco Co., U.S.A.

1.2.2 Methods

(i) Preparation of the dispersion media

In addition to the FCO alone, the following three types of systems were investigated in the assessment of the rheological behaviours of 24 different vehicles.

Type 1 vehicles

Vehicles of this type relate to that described by Stephens and Su (1975).

(a) 0.5% w/v, 1% w/v, 1.5% w/v, 2% w/v, 2.5% w/v, 3% w/v, 3.5% w/v, 4% w/v or 5% w/v of a 50:50 mixture of aluminium mono- and distearate dispersions in FCO.

(b) solution of 0.7% w/v lecithin in FCO.

(c) dispersion of 0.35% w/v of hydrogenated castor oil in FCO.

(d) dispersion of 0.5% w/v aluminium stearate + 0.7% w/v lecithin + 0.35% w/v hydrogenated castor oil + 20% w/v sucrose in FCO.

(e) dispersion of 0.5% w/v aluminium stearate + 0.35% w/v hydrogenated castor oil + 20% w/v sucrose in FCO.

(f) dispersion of 0.5% w/v aluminium stearate + 0.7% w/v lecithin + 0.35% w/v hydrogenated castor oil + 30% w/v sucrose in FCO.

Vehicles d and f were prepared according to the patent of Stephens and Su (1975) by dissolving the lecithin in a portion of the FCO. Dissolution was facilitated by heating the FCO to about 90-100^{o}C and agitating the mixture thoroughly until all the solids were dissolved. To this solution, with the heat maintained, the aluminium stearate and hydrogenated castor oil were added and the resulting mixture was mixed well until the latter two ingredients were thoroughly dispersed. Then the sucrose, previously sieved to a mesh size of 63-75µm, was added and the resulting dispersion was mixed thoroughly with the temperature at 90-100^{o}C for 3 hrs.

The resulting dispersion was cooled to room temperature with mixing. The remainder of the FCO was then added to bring the dispersion up to the volume. Care was taken to avoid the entry of any moisture into the container, since preliminary studies showed

that water affects the structure of the gel. The remaining vehicles (a), (b), (c) and (e) were prepared in accordance with appropriate stages in the above method. For example, vehicles of type 1(a) were prepared simply by adding the required amounts of aluminium stearate to a portion of the FCO in a flask and heating in a water bath (90-100)^{o}C for 3 hrs with thorough agitation. For vehicles (b) and (c) the required amount of the ingredient was added to a portion of the oil and mixed thoroughly at a temperature of 90-100^{o}C until the solid was dissolved in the case of (b) or thoroughly dispersed in (c). Vehicles (a) (b) and (c) were then cooled to room temperature and the remainder of the oil was added as mentioned above.

Type 2 vehicles

Vehicles of this type relate to that described by Lin and Pramoda (1978).

(a) dispersions of (i) 20% w/v or (ii) 30% w/v of sucrose in FCO.

(b) (i) 0.3% w/v, (ii) 0.5% w/v or (iii) 1% w/v of Cab-o-sil in a 20% w/v dispersion of sucrose in FCO.

(c) 1.25% w/v Cab-o-sil + 30% w/v sucrose in FCO.

(d) 1% w/v Cab-o-sil in FCO.

Vehicles (b) and (c) were prepared according to the patent of Lin and Pramoda (1978) by adding the sugar in successive portions to a portion of the FCO in a suitable container and stirring until the system was suitably dispersed and suspended. The Cab-o-sil was then added and stirred until dispersed. Sufficient additional oil was added and stirred to obtain a uniform dispersion. Although it was not specified in this patent, the sucrose was sieved and the portion corresponding to a mesh size of 63-75 μm was used in the preparation of the vehicle. Precautions were taken to avoid the entry of moisture into the container for the same reason mentioned above.

The vehicles that contain only dispersions of sucrose in the oil (a) or Cab-o-sil in the oil (d) were also prepared according to this patent simply by adding the sucrose or Cab-o-sil to the oil and stirring until complete dispersion was obtained.

Type 3 vehicles

These aqueous vehicles were included for use in comparative in vivo and in vitro studies.

(a) 0.25% w/v xanthan gum in distilled water.

(b) 0.25% w/v xanthan gum + 20% w/v sucrose in distilled water.

(c) 30% w/v sucrose in distilled water.

100 cm^3 quantities of the dispersions were prepared by wetting 0.25 gm of gum with 2 cm^3 of 90% v/v alcohol and then adding 75 cm^3 of distilled water or 20% w/v sucrose solution to prepare (a) and (b) respectively. The dispersions were allowed to hydrate for 24 hrs. The volumes were made up to 100 cm^3 with appropriate vehicle.

All vehicles of every type (except 3c) were then homogenised for one minute using an Ultra-Turrax mixer at a fixed speed and stored overnight. On the following day the dispersions were stirred gently and warmed to 37°C before use.

(ii) Rheological measurements

A Rotovisko viscometer (Haake) fitted with concentric cylinder sensors and a temperature controlled water jacket at 37°C was used. The NV measuring cup and bob with measuring head 500 were selected; this combination was suitable for the viscosity range studied.

The measuring cup was filled with a dispersion, previously warmed to 37°C, and the bob immersed. The dispersion was allowed to remain undisturbed for 2 min to allow temperature equilibrium to be re-established. The bob was set in motion at the lowest shear rate, and a reading was taken at the end of 30 seconds. The rotational speed

of the bob was then increased to the next setting at 30 second intervals until the highest shear rate was reached, then decreased at the same rate to the lowest shear rate and scale readings were recorded at the end of each 30 seconds period. The total time for each measuring cycle was therefore 5 minutes.

1.3 Results

The rate of shear (D) that is applied to the system under test at a given speed of rotation of the bob in the Rotovisko viscometer is given by Eq. 1.2

$$D = \frac{B}{U} \; s^{-1} \qquad \text{Eq. 1.2}$$

where B is a constant that depends on the dimensions of the concentric cylinders and U is a speed factor.

The scale readings (S) observed on the instrument can be converted into shear stress (τ) values by Eq. 1.3

$$\tau = AS \qquad N\,m^{-2} \qquad \text{Eq. 1.3}$$

where A is a constant (the stress factor) that depends on the dimensions of the sensor system that is used and on the torsional constant of the instrument.

Tables of results that listed the values of the instrument parameters, observed scale readings and derived shear rates and stresses were prepared for each system that was studied. Table 1.1 is an example of such a table and shows the results obtained for FCO.

Table 1.1 Rheological parameters for FCO at 37°C

Sensor - NV, Measuring head - 500

U	S	D(s^{-1})	τ(N m^{-2})
162	-	-	-
81	0.31	32	0.6
54	0.50	49	0.9
27	1.00	97	1.8
18	1.38	146	2.5
9	2.75	291	5.0
6	4.25	436	7.8
3	8.38	873	15.3
2	12.75	1310	23.3
1	25.00	2620	45.8

Rheograms or flow curves, i.e. plots of shear rate versus shear stress, for all the systems studied in this chapter are shown in Fig. 1.1 - 1.4. These figures describe the "upcurves" only and extend to a maximum shear rate of 436 s^{-1} for the sake of clarity at the lower shear rates. With the exception of the linear (i.e. Newtonian) flow curves that were obtained for FCO alone (curve(1) in Fig. 1.1), 0.7% w/v lecithin in oil (curve 1(b) in Fig. 1.2) and 30% w/v sucrose in water, the remaining rheograms indicated varying degrees of pseudoplastic behaviour of the oily dispersions referred to in Fig. 1.1 - 1.3 and the aqueous systems referred to in Fig. 1.4. In addition to pseudoplastic behaviour, systems containing concentrations of aluminium stearate greater than 1% w/v in FCO also exhibited a slight thixotropy. The complete rheograms (i.e. upcurves and downcurves) for 1% w/v and 5% w/v aluminium stearate in FCO over the complete range of shear rates that was used and that for vehicle type 1(f), which corresponds to the vehicle described in Stephens and Su's patent (1975), are shown in Fig. 1.5 and 1.6 respectively, in order to illustrate the hysteresis loops that denote thixotropic behaviour in these systems.

The apparent viscosities at an arbitrarily chosen low shear rate of 100 s^{-1} of a series of aqueous vehicles have been correlated with the bioavailabilities and *in vitro* release rates of a series of drugs (Barzegar-Jalali and Richards, 1979 a and b). The apparent viscosities of the systems used in the present work were calculated at the same shear rate and their values are listed in Table 1.2.

Table 1.2 Apparent viscosities (η_{app}) of the vehicles at a shear rate of 100 s^{-1} and temperature of 37°C

The Vehicle	η_{app} mN s m^{-2}
Distilled water (D.W.)	0.695 (a)
30% w/v sucrose in D.W.	2.32
Fractionated Coconut Oil (FCO)	17.5
0.7% w/v lecithin solution in FCO	23
0.25% w/v xanthan gum in D.W.	33
0.5% w/v aluminium stearate in FCO	37
0.25% w/v xanthan gum + 20% w/v sucrose in D.W.	38
0.35% hydrogenated castor oil in FCO	40
1% w/v aluminium stearate in FCO	50
20% w/v sucrose in FCO	51
1% w/v Cab-o-sil in FCO	58
1.5% w/v aluminium stearate in FCO	59
30% w/v sucrose in FCO	64
2% w/v aluminium stearate in FCO	69
2.5% w/v aluminium stearate in FCO	81
0.3% w/v Cab-o-sil + 20% w/v sucrose in FCO	83
3% w/v aluminium stearate in FCO	92
0.5% w/v Cab-o-sil + 20% w/v sucrose in FCO	98
3.5% w/v aluminium stearate in FCO	104
0.5% w/v aluminium stearate + 0.35% w/v hydrogenated castor oil + 20% w/v sucrose in FCO i.e. type 1 vehicle e (1e)	105
vehicle (1e) + 0.7% w/v lecithin i.e. (1d)	120
1% w/v Cab-o-sil + 20% w/v sucrose in FCO	131
vehicle (1d) using 30% w/v sucrose i.e. vehicle (1f)	140
4% w/v aluminium stearate in FCO	144
1.25% w/v Cab-o-sil + 30% w/v sucrose in FCO	150
5% w/v aluminium stearate in FCO	176

(a) Liley et al (1963).

Fig.1.1 Rheograms of FCO and the dispersions of aluminium stearate in FCO (Type 1a vehicles).

(i) FCO
(ii) 0.5% w/v aluminium stearate in FCO
(iii) 1% w/v .. .. ..
(iv) 1.5% w/v .. .. ..
(v) 2% w/v .. .. ..
(vi) 2.5% w/v .. .. ..
(vii) 3% w/v .. .. ..
(viii) 3.5% w/v .. .. ..
(ix) 4% w/v .. .. ..
(x) 5% w/v .. .. ..

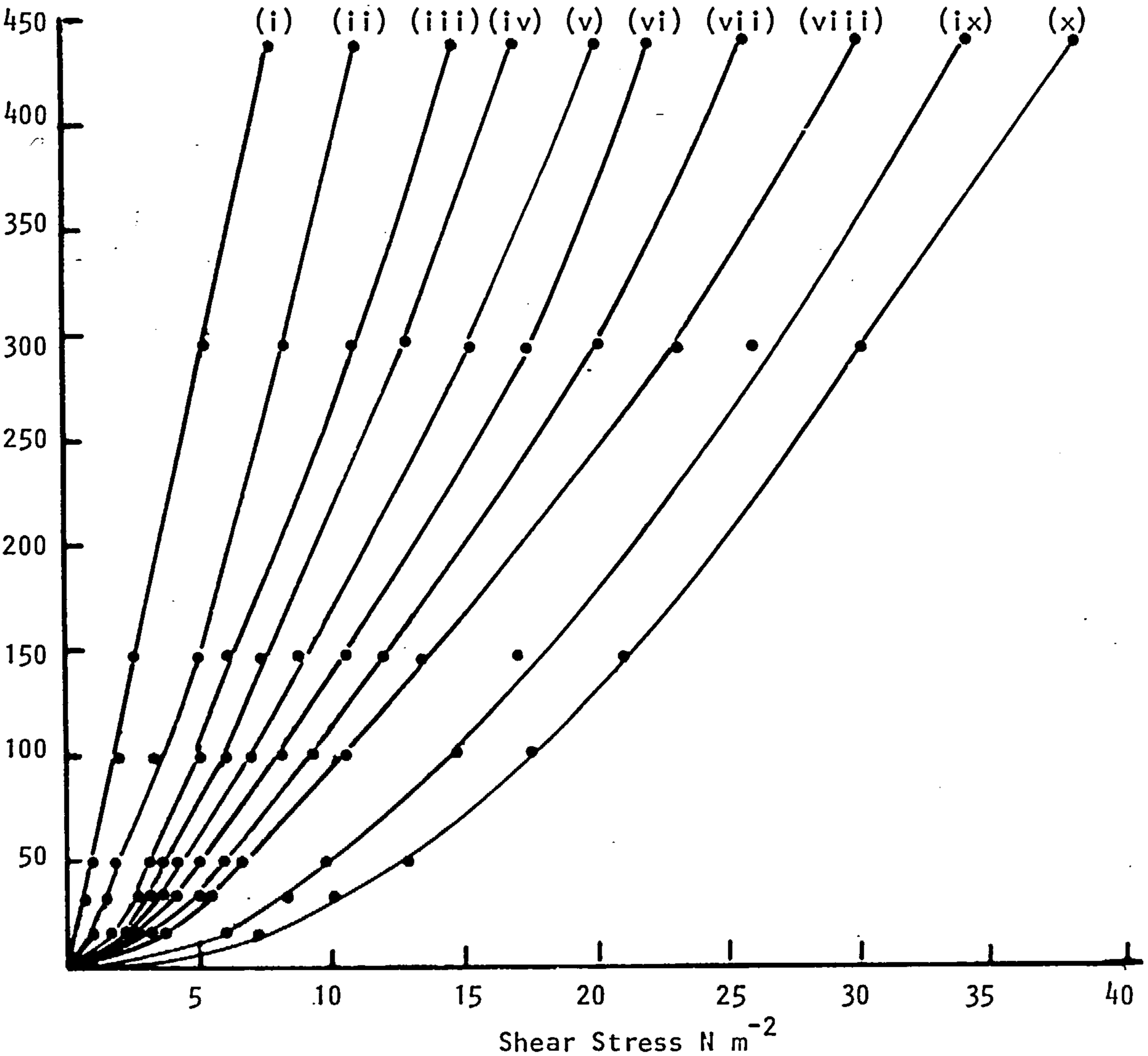


Fig 1.2 Rheograms of oily vehicles Type 1(b)-(f) and Type 2 (a) at 37°C.

Key: see pages 71 and 72.

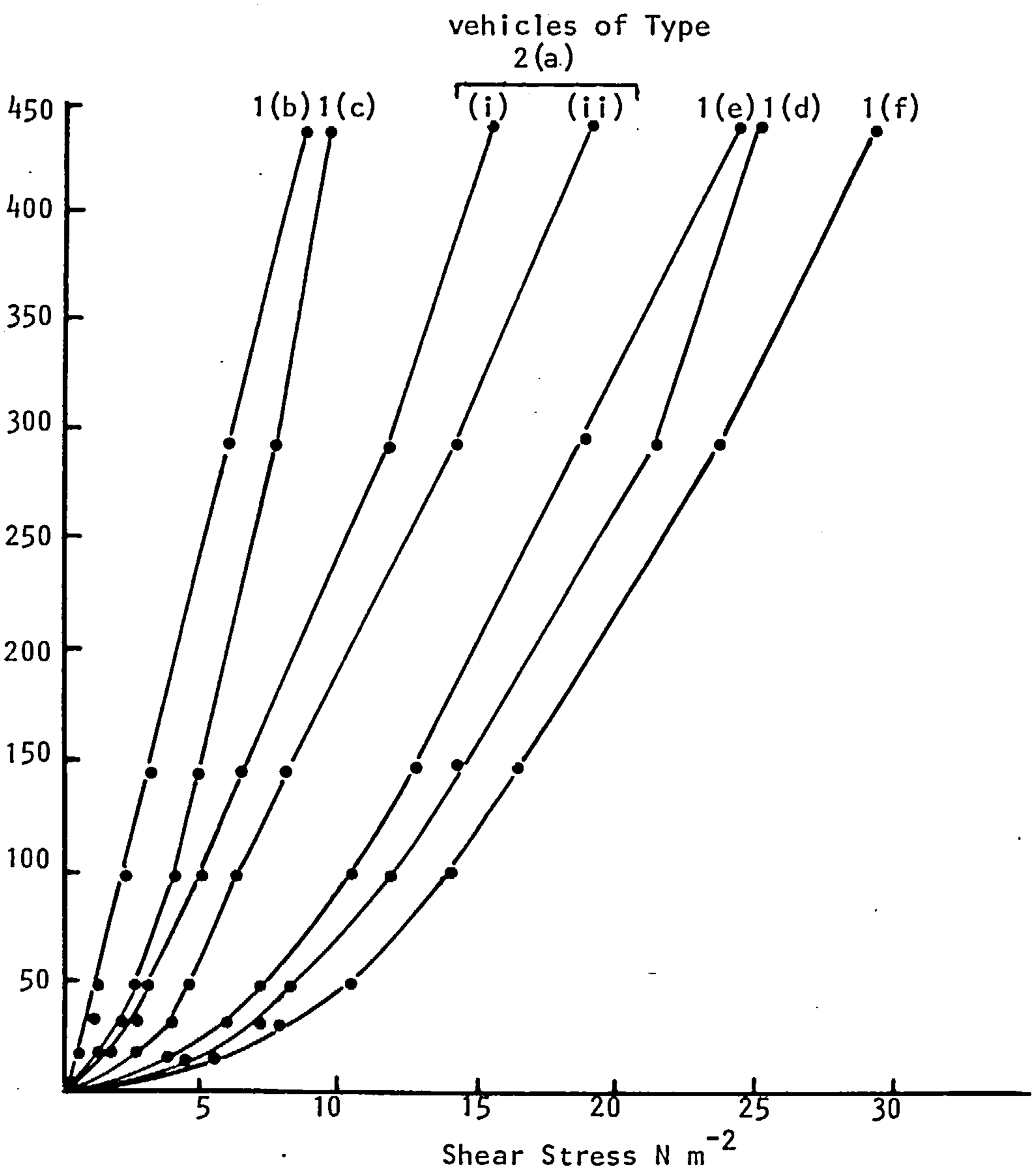


Fig. 1.3 Rheograms of oily Type 2(b)-(d) vehicles at 37°C

Key: see page 72.

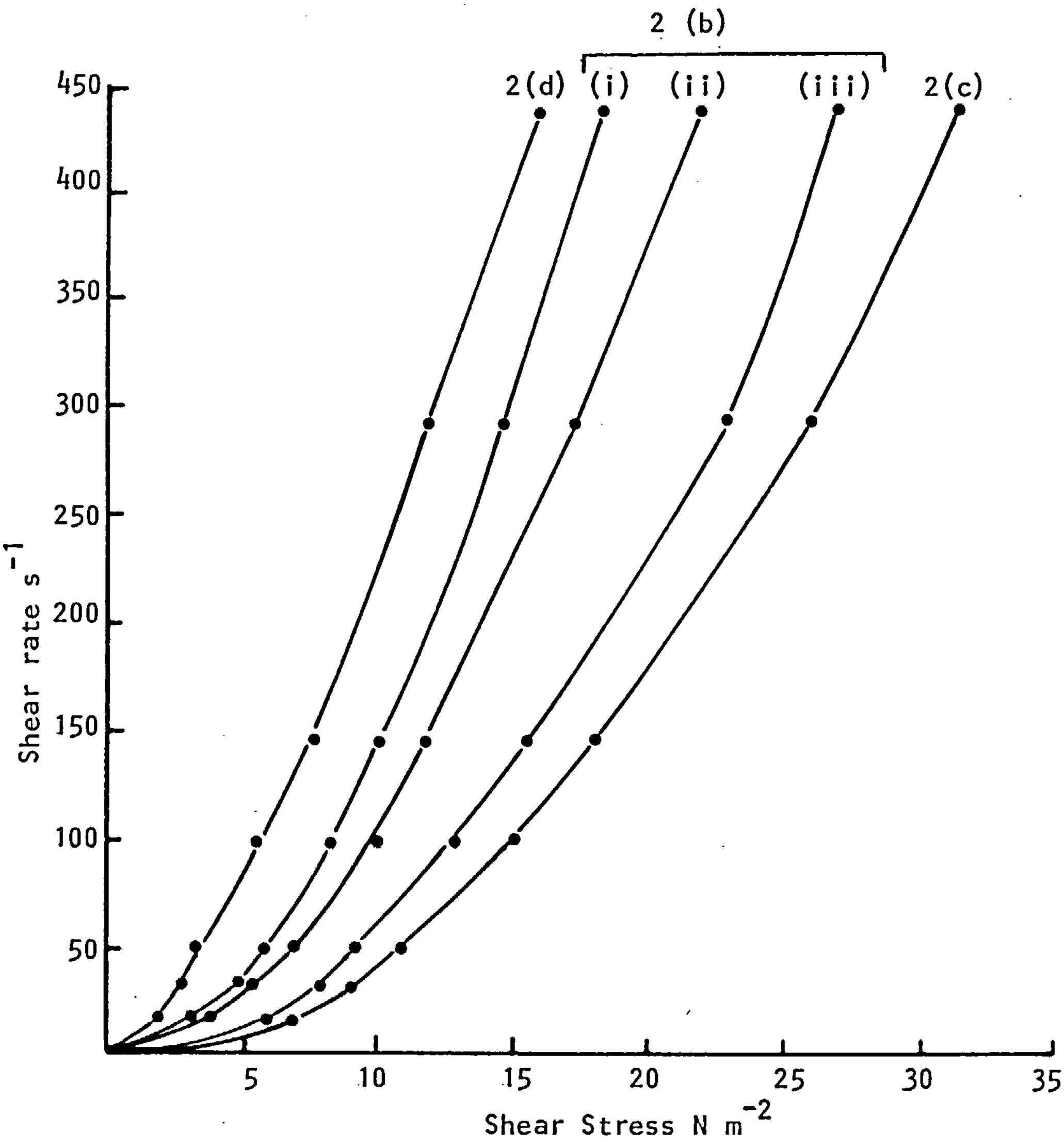


Fig.1.4 Rheograms of aqueous Type 3 vehicles at 37°C

Key: see page 73.

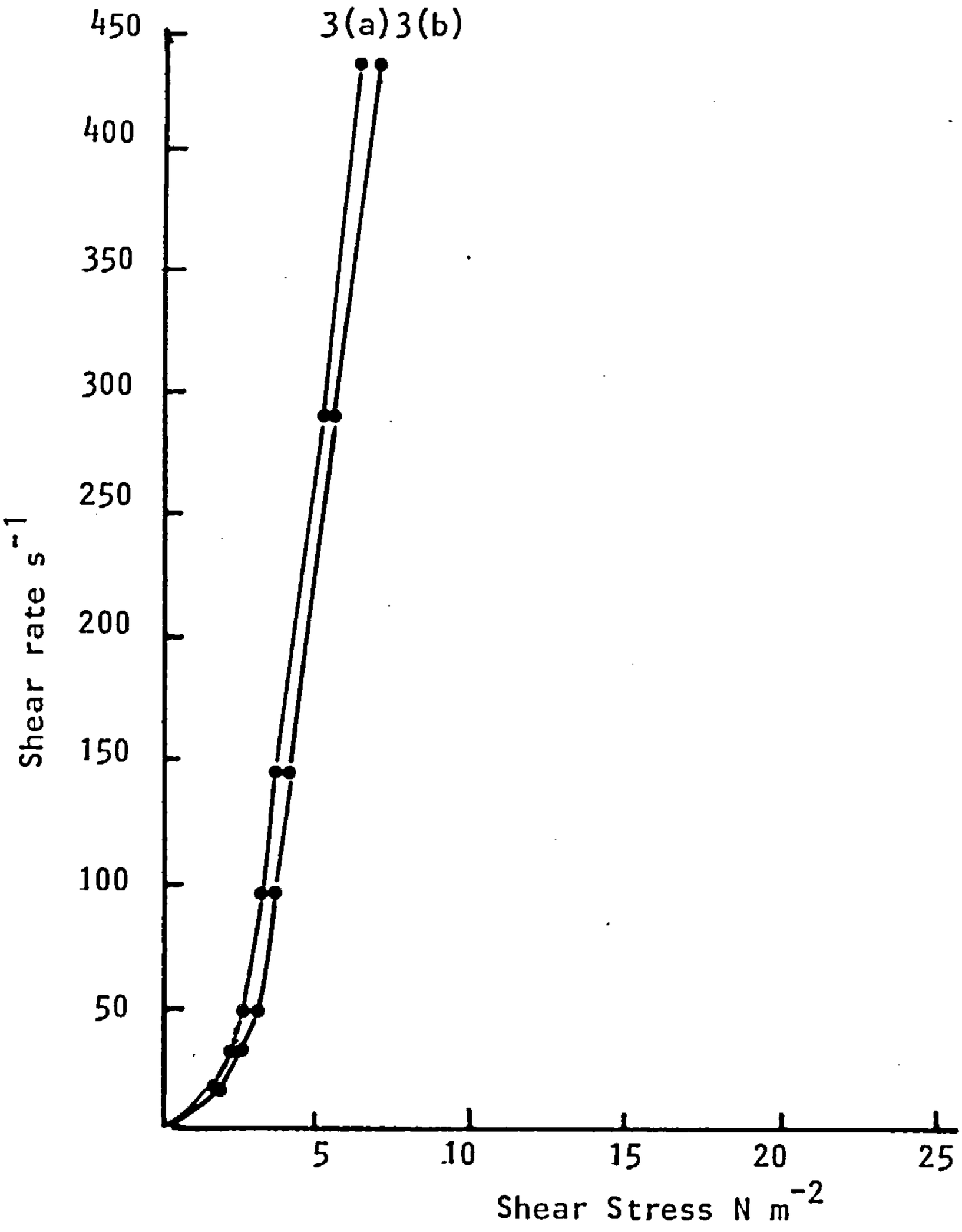


Fig. 1.5 Rheograms of oily dispersions of 1% w/v and 5% w/v aluminium stearate in FCO showing hysteresis loop.

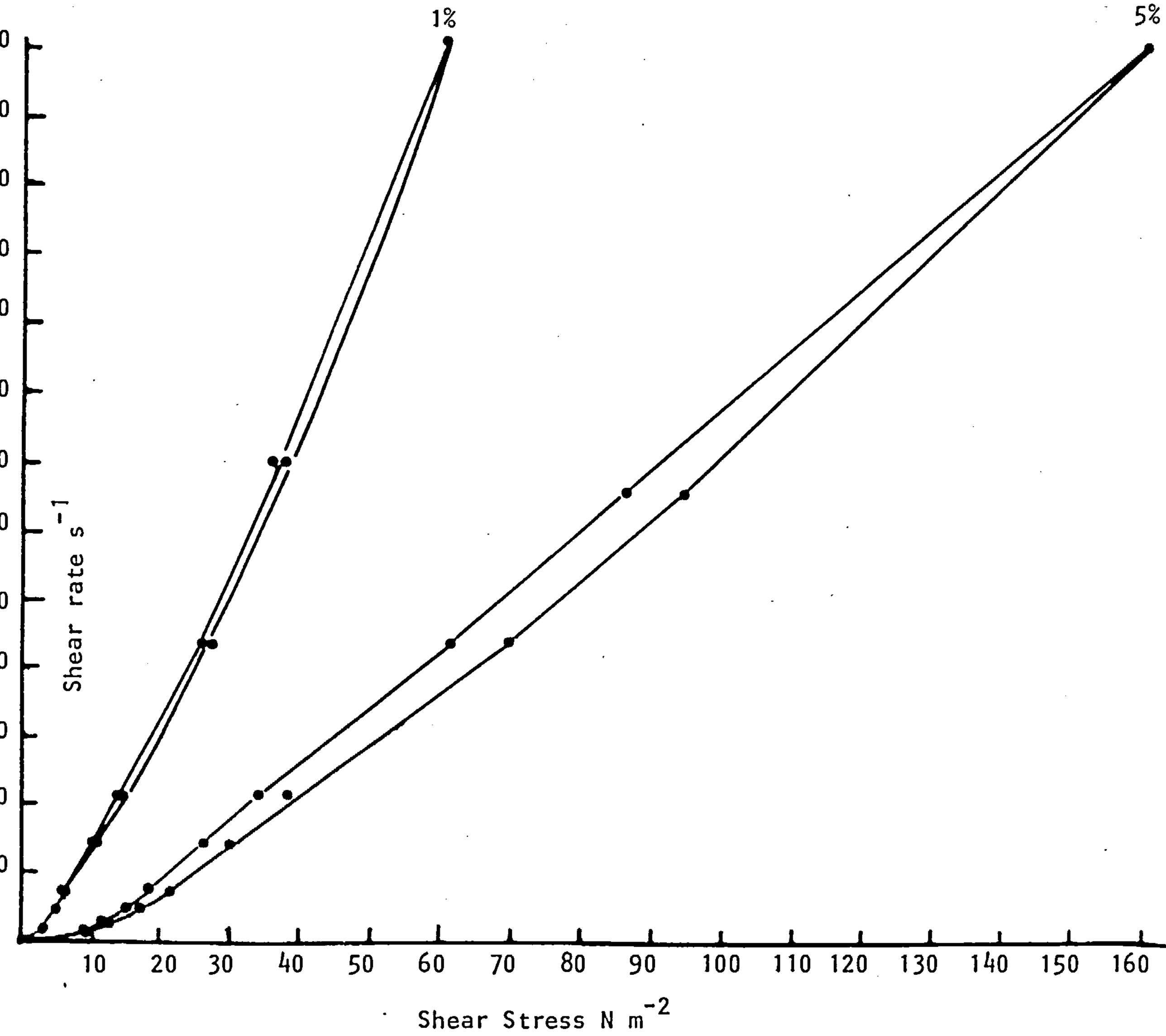
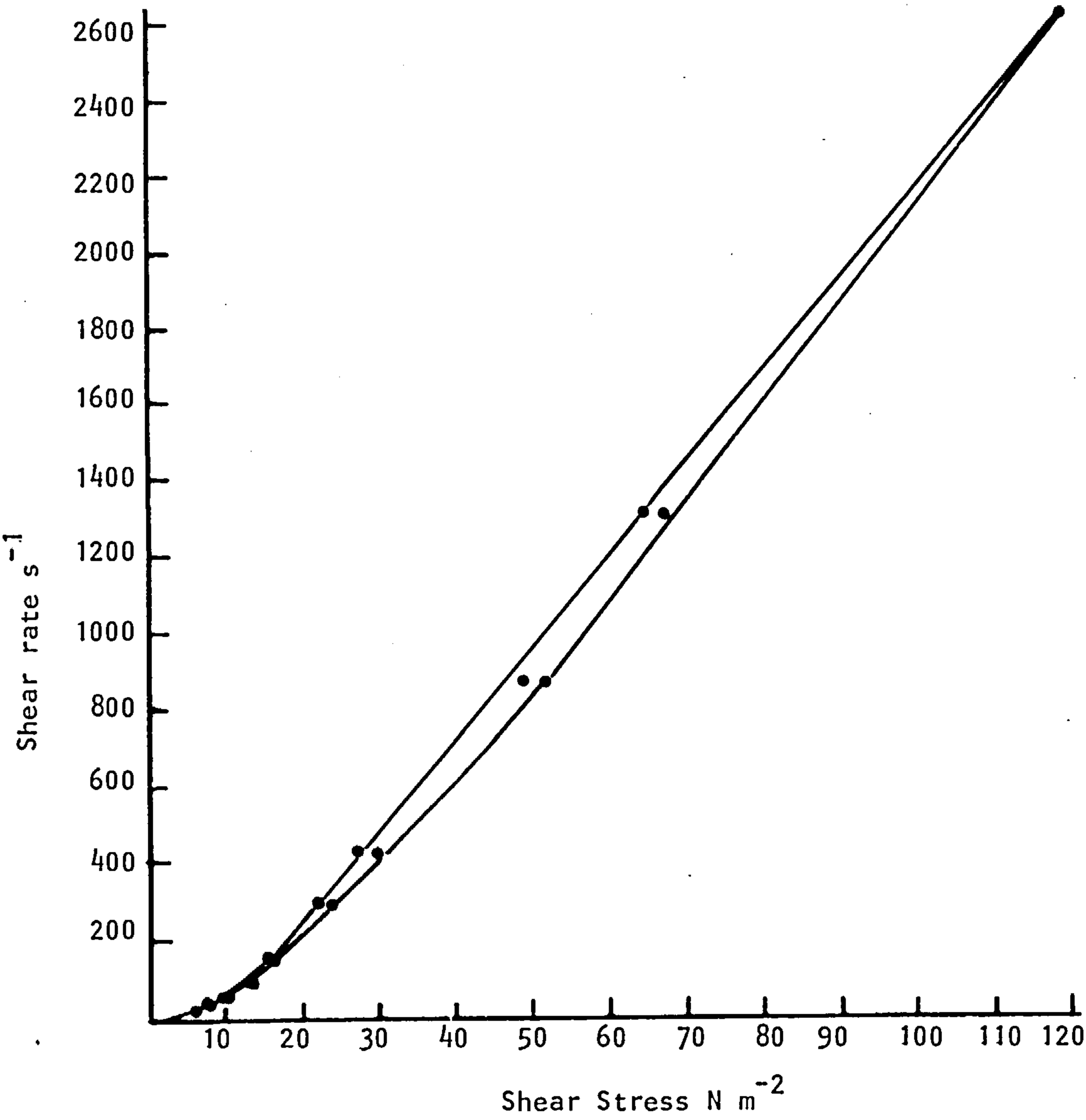


Fig. 1.6 Rheogram of vehicle Type 1(f) showing hysteresis loop.



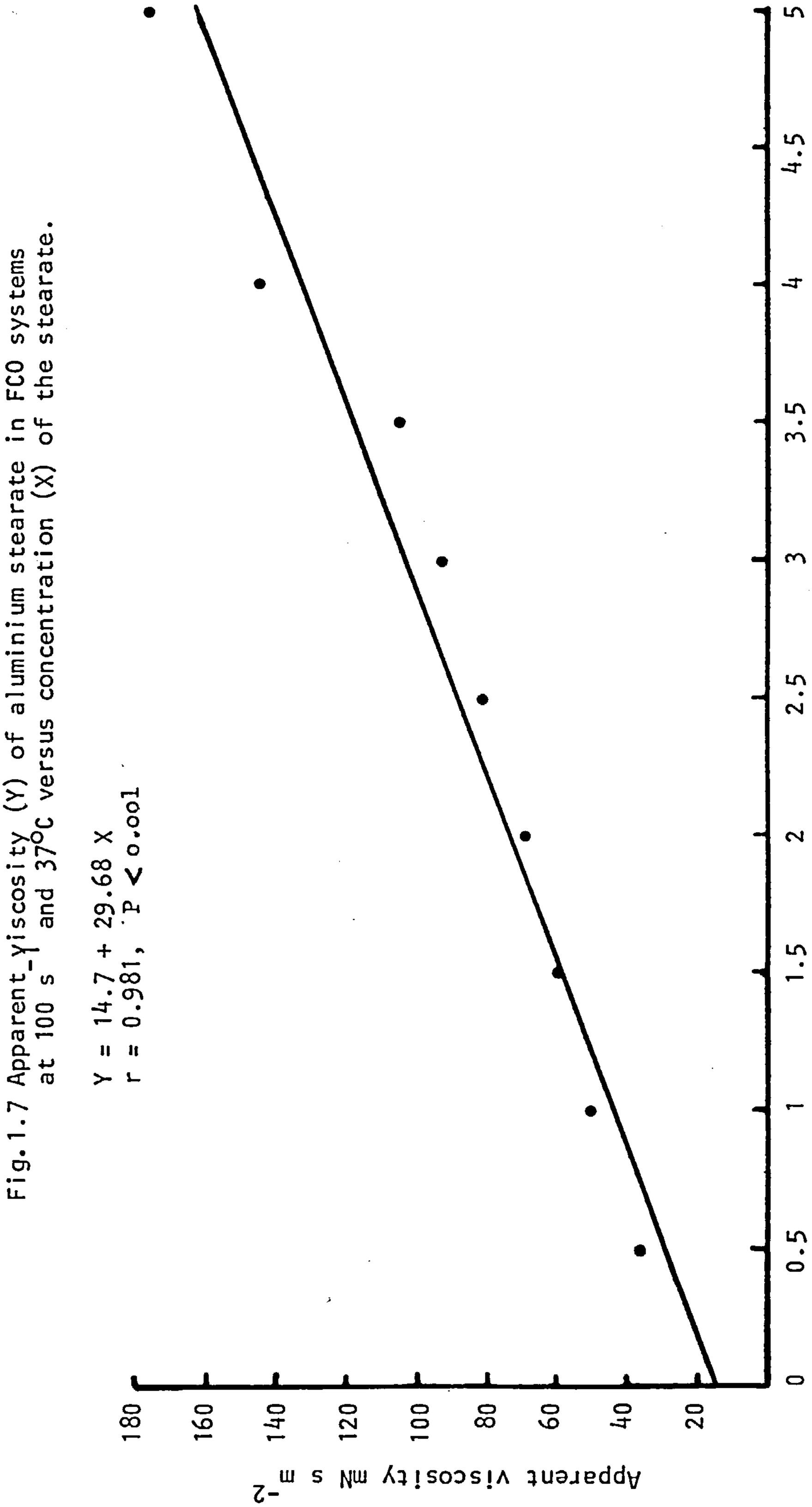


Fig. 1.7 Apparent viscosity (Y) of aluminium stearate in FCO systems at 100 s^{-1} and 37°C versus concentration (X) of the stearate.

$Y = 14.7 + 29.68\ X$

$r = 0.981,\ P < 0.001$

1.4 Discussion

The Newtonian behaviour exhibited by FCO, 0.7% w/v lecithin solution in the oil and the syrup, i.e. 30% w/v sucrose in distilled water is as expected for simple liquids and true solutions (Martin et al, 1964 and 1973). Dispersions of aluminium stearate, hydrogenated castor oil, Cab-o-sil and sucrose in FCO, together with those oily vehicles specified by the patents of Stephens and Su (1975) (type 1 vehicles, d and f) and Lin and Pramoda (1978) (type 2 vehicles, b and c), exhibited pseudoplastic behaviour. These findings are in agreement with those reported in the literature concerning Cab-o-sil (Lin and Pramoda, 1978), and sucrose(Martin et al, 1964 and 1973). Furthermore, aluminium stearate, 1% w/v and above, and the oily vehicle type 1 (d and f) exhibited thixotropic pseudoplastic behaviours. The thixotropic property of oily gels of aluminium stearate has been reported previously (Buckwalter and Dickison, 1948 and 1958; Phake, 1975).

When classifying materials according to the types of flow and deformation, it is customary to place them in one of two categories; i.e. Newtonian or Non-Newtonian systems. The choice depends on whether or not their flow properties are in accordance with Newton's law of flow (Martin et al, 1973). According to this law the higher the flow resistance or viscosity of a liquid the greater is the force required to cause the liquid to flow. The force per unit area, τ, imposed on the liquid is called the shearing stress. The velocity gradient of the liquid produced by this force is referred to as the rate of shear, D.

In Newtonian systems the vehicle exhibits a constant proportionality between (τ) and (D) and consequently obeys Newton's

law, which is expressed by Eq. 1.4 or 1.5

$$\tau = \eta D \qquad \text{Eq 1.4}$$

$$\text{or} \qquad \eta = \frac{\tau}{D} \qquad \text{Eq 1.5}$$

where η is the coefficient of viscosity, ordinarily called by the shortened term, viscosity. The SI unit of viscosity is $N\ s\ m^{-2}$ Examples of Newtonian fluids include water, glycerin and true solutions, such as syrup (Martin et al, 1964 and 1973). Their viscosities do not depend on shear stress or rate of shear. The flow curves or rheograms of Newtonian liquids are straight lines which commence at the origin. Viscosity is the reciprocal of the slopes of such a line (D vs τ) or the cotangent of the angle it makes with the horizontal axis (Martin et al, 1973).

The addition of dispersed particles and/or suspending agents to a Newtonian vehicle often produces non-Newtonian properties. For example, the addition of the suspending agent xanthan gum to the water (Fig.1.4) or aluminium stearate (Fig.1.1), hydrogenated castor oil (curve 1(c) in Fig.1.2), sucrose (curves 2(a) in Fig.1.2) and Cab-o-sil (Fig.1.3) to the oil produced non-Newtonian systems.

Non-Newtonian flow behaviours do not follow Newton's law, i.e. Eq. 1.4. The non-Newtonian property may be plastic, pseudoplastic or dilatant behaviour. These behaviours may be either time independent or time dependent, i.e. thixotropic. Dilatant and plastic behaviours are not encountered in this study, therefore, only pseudoplasticity and thixotropic pseudoplasticity will be discussed here.

Unlike systems that exhibit plastic behaviour pseudoplastic materials do not possess a yield value, but instead are characterised

by rheograms which begin at the origin, (or at least approach it at low rate of shear), as in the case of Newtonian liquids. However, unlike the curve for a Newtonian material, the pseudo-plastic flow curve is not linear, as shown by nearly all of the rheograms in Fig.1.1 - 1.4. Another way of expressing this fact is to say that the shear stress, τ, does not increase linearly with the shear rate, D, i.e. pseudoplasticity involves a decrease in the proportionality factor of shear stress: shear rate with increase in shear stress so that the viscosity apparently decreases as the shear stress or shear rate increases. This behaviour cannot be expressed quantitatively by fundamental equations but an empirically derived equation (Eq. 1.6) is often applicable (Krieger and Maron, 1951; Kabre et al, 1964).

$$F^n = \eta' D \qquad \text{Eq. 1.6}$$

when n and η' are constants for a given system. n is greater than 1 and the higher the value of n the greater the degree of pseudoplasticity. The viscosity coefficient η', that is defined in Eq. 1.6, is a constant for a particular material, but, unlike Newtonian viscosity η, it is difficult to assign any physical meaning to it. (Note. an equation similar to Eq. 1.6 may be used to describe the flow of dilatant systems, which exhibit behaviour opposite to that of pseudoplastic systems; i.e. η_{app} increases with increase in shear rate. In these cases the constant n in Eq. 1.6 is less than 1).

Since the viscosity of a pseudoplastic substance decreases with increasing rate of shear, an apparent viscosity, η_{app} is commonly used to denote the viscosity of the system at a particular shear stress or shear rate. It can be expressed as either the reciprocal

slope of a line joining the appropriate point on the flow curve with the origin of the graph (Green, 1949; Martin et al, 1973) or as cotangent to the flow curve at the specified point (Fischer, 1950; Martin et al, 1973). The former method has been used to express the apparent viscosity of the vehicle dispersions at a shear rate of 100 s^{-1} throughout this study.

The decrease in the η_{app} with increasing shear results from the breakdown, under the influence of shearing force, of structures within the system. The structural features of these systems involve gel formation, e.g. aluminium stearate (Shiba, 1960; Stephens, 1971) and Cab-o-sil in the oil (Sherriff and Enever, 1979); or the intertwining of macromolecules and entrapment (immobilisation) of solvent molecules within the entanglements as in an aqueous dispersion of xanthan gum (Martin et al, 1964 and 1973). Breakdown of these structures occurs under the influence of shear, i.e. gel networks are disrupted or macromolecules tend to become aligned with their long axes parallel to the direction of flow so that intermolecular intertwinings are reduced and entrapped solvent molecules are released. The viscosity of the system consequently decreases with increase in the rate of shear. For this reason pseudoplastic materials are sometimes called "shear thinning systems". When structural breakdown is complete the η_{app} becomes constant, i.e. further increase in shear rate will not cause any additional decrease in viscosity. On removal or reduction of the shear force reformation of the structural units occurs under the influence of Brownian motion. If this reformation occurs immediately, the flow curve obtained at decreasing shear rates (the downcurve) is therefore superimposable on that obtained from measurements made at increasing shear rates (the upcurve). This is the case with the oily dispersions of sucrose

(curves 2(a) in Fig.1.2), Cab-o-sil alone (curve 2(d) in Fig.1.3)and with sucrose (curves 2(b) and (c) in Fig.1.3) and aqueous dispersions of xanthan gum (Fig.1.4). This type of pseudoplastic behaviour is called time independent pseudoplastic behaviour.

However, there are instances where the downcurve is not superimposable on the upcurve, i.e. the reformation of structure is not immediate when the stress is removed or reduced, and the downcurve is therefore displaced to the left with regard to the upcurve. For this reason such flow behaviour is called time dependent or thixotropic pseudoplasticity (Martin et al, 1964 and 1973).

Thixotropy, therefore, may be defined as "an isothermal and comparatively slow recovery, on standing of a material, of a consistency lost through shearing" (Martin et al, 1973). This is due to the fact that if the suspension is viscous or the particles are large and heavy their Brownian motion is too slow to restore broken interparticular links or to regain the former state of entanglement of macromolecules "instantaneously". If the rate of link restoration by Brownian motion is lower than the rate of link breakdown by shear the η_{app} decreases even while the system is under constant shear.

The extreme behaviour is an isothermal, reversible sol $\rightleftharpoons$ gel transformation produced by shear and by rest, respectively. For example, higher concentrations of aluminium stearate, e.g. 4% w/v and 5% w/v (Fig.1.5), and the oily vehicle (type 1 vehicles d and f) (Fig.1.6), form gels after preparation when unstirred, but flow and can be poured more easily after they had been stirred vigorously. After a period of about 1 hour they revert to gels as the Brownian motion rebuilds their gel structure.

It has been suggested that the area enclosed by the hysteresis loop in the rheogram of a thixotropic system can be used as a relative measure of the degree of thixotropic breakdown. In addition, thixotropy can be represented quantitatively by the decay of shear stress or apparent viscosity as a function of time at a constant rate of shear (Fischer, 1950; Martin et al, 1964 and 1973) or by a coefficient of thixotropic breakdown, i.e. the loss in shearing stress per unit increase in shear rate (Martin et al, 1973). Using the trapezoidal rule that is commonly used in bioavailability studies, the areas of the hysteresis loops of the oily vehicles (type 1 vehicles, d and f) and the oily dispersions of aluminium stearate systems were calculated by subtracting the smaller area under the curve from the larger one. These areas (thixotropic indices) are shown in Table 1.3.

Fig. 1.5 illustrates the thixotropic nature of oily dispersions of 1% w/v and 5% w/v aluminium stearate. The hysteresis loops obtained for other intermediate concentrations lie between these two curves but have been omitted from the figure for the sake of clarity. However, their thixotropic indices are given in Table 1.3. Fig. 1.5 and Table 1.3 show that the degree of thixotropy increases with increase in the aluminium stearate concentration. The apparent viscosity at a given shear rate also increases in an approximately linear manner as indicated by Fig. 1.7.

There is a disagreement in the literature concerning the time dependency of the flow behaviours of aluminium soap-hydrocarbon systems. Complete recovery was reported by Goldberg and Sandvick (1947), and Carver and Van Wazer (1947). However, Evans and Matthews (1954) and Shiba (1960) stated that the change caused by the applied shearing stress is not thixotropic but appears to be a permanent change in the

Table 1.3 Thixotropic indices of the oily vehicles that formed hysteresis loops

Vehicle Type	Concentration of aluminium stearate	Thixotropic index $N\ s^{-1}m^{-2}\ 1 \times 10^3$
1a	1%	1.433
	1.5%	2.224
	2%	3.662
	2.5%	5.697
	3%	7.141
	3.5%	10.632
	4%	11.705
	5%	13.840
1d		4.549
1f		4.826

structure of the gel. Although no evidence of recovery was observed with a 9% w/w gel gradual recovery did occur with a 3% w/w and this latter system was therefore thixotropic (Stephens, 1971). The author suggested that recovery may, in fact, occur with the 9% w/w gel, but at an extremely slow rate due to the viscosity of the system.

However, the discrepancies between the results obtained by different workers can be ascribed mainly to differences in the experimental conditions (Shiba, 1960); for example, the rate of shear, temperature, concentration and the properties of aluminium stearates, which are not always pure substances. Although the above discrepancies have been reported, Shiba (1960) thought that the paraffin gels were thixotropic at high temperature or under low rates of shear.

On the basis of differences in experimental conditions and nature of the oily phase it is possible, therefore, to explain why aluminium stearate exhibited pseudoplastic flow behaviour in the FCO in this study, whereas plastic behaviour in liquid paraffin was reported previously (Shiba, 1960; Stephens, 1971). Furthermore, these differences are also likely to be responsible for the fact that 0.5% w/v aluminium stearate produced a gel in FCO (curve 2 in Fig.1.1) and 1% w/v produced a gel with thixotropic properties (Fig.1.5) whereas 1.5% w/w aluminium stearate appeared to be required in liquid paraffin to produce the same sort of effects (Stephens, 1971).

Dispersion of sucrose (20% w/v and 30% w/v) in FCO also exhibited non-Newtonian properties, but these were limited to pseudoplastic behaviour since no evidence of thixotropy was observed.

In systems containing 20% w/v sucrose plus different concentrations of Cab-o-sil the pseudoplastic viscosity increased with increasing

concentration of Cab-o-sil (curves 2(b) in Fig. 1.3). Pseudoplasticity was also exhibited by 1.25% w/v Cab-o-sil plus 30% w/v sucrose as well as by 1% w/v Cab-o-sil in the oil alone (curves 2(c) and 2(d) in Fig. 1.3 respectively). The effects of Cab-o-sil have been reported by Lin and Pramoda (1978). The action of Cab-o-sil as a viscosity enhancing agent may be largely attributed to the ability of the very small silica particles to form a network structure throughout the medium by interparticular hydrogen bonding via the silanol groups on the silica surface. In addition to these particle interactions, there is possible bonding between the silanol groups and other components that are also capable of hydrogen bond formation (Marshall and Rochester, 1975). No thixotropic properties were detected in the Cab-o-sil systems and it is suggested that the recovery and reformation of the structural units, upon removal or reduction of shear force, occurs immediately under the influence of Brownian motion.

The effects of the other pharmaceutical additives that are included in type 1 vehicles on the rheological properties of the oil are shown in Fig. 1.2 (curves 1(b),(c),(d),(e) and (f)). A 0.7% w/v solution of lecithin in FCO was still Newtonian although the viscosity was increased slightly as indicated in Table 1.2.

The omission of 0.7% lecithin from type 1 vehicles d and f resulted in a decrease in the apparent viscosity as shown by Table 1.2 and the loss of the thixotropy that is exhibited by vehicles of type 1 (f and d). Thus the thixotropic structure in these latter vehicles depends on the presence of lecithin, although on its own in FCO this compound appears to act as a simple Newtonian solution. The tendency for lecithin to form reasonably stable complexes with other substances, especially other lipids, proteins and carbohydrates,

provides a probable explanation for the formation of a thixotropic system (West et al, 1966). Thus the type 1 vehicles (d and f), that are described by the patent of Stephens and Su (1975), probably consist of dispersion of sucrose and hydrogenated castor oil particles that are coated with adsorbed aluminium stearate and lecithin molecules and the thixotropic structure results from interparticular bridges formed by the combined effects of lecithin and aluminium stearate when their concentrations are adequate. Alternatively, or additionally, the lecithin and stearate may, if their combined concentrations are sufficient, form a gel network through the system and the insoluble sucrose and hydrogenated castor oil particles are simply suspended in this network.

SECTION 3

IN VIVO STUDIES

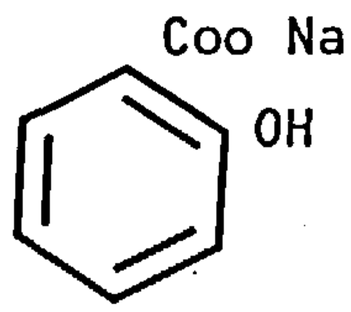
CHAPTER 1

A COMPARATIVE BIOAVAILABILITY STUDY ON AN AQUEOUS SOLUTION AND AN OILY SUSPENSION OF SODIUM SALICYLATE IN THE RABBIT

1.1 Introduction

(a) Physico-chemical properties

Sodium salicylate is the sodium salt of a weak acid (salicylic acid,SA) with a pK_a of 3. It has the following chemical structure:



and exists as almost odourless, colourless, small crystals or shiny flakes, or as a white crystalline powder, with a salty-sweet taste. It is soluble at 20°C in 1 part of water and in 11 parts of alcohol (British Pharmaceutical Codex, 1973).

Munzel (1971) pointed out that "the most effective means of attaining high dissolution rates is to use a highly water-soluble salt of a weak acid instead of the free acid itself. The dissolution rate of the sodium salt of a weak acid in an acidic dissolution medium under certain circumstances may be 1000 times higher than the dissolution rate of the weak acid itself. Even if the free acid precipitates subsequently from the sodium salt in the bulk phase of an acidic solution such as gastric fluid, it will do so usually in the form of very fine particles. The large surface area of the drug thus precipitated favours rapid dissolution as additional fluid becomes available or as some of the dissolved drug is removed by absorption".

(b) Action and uses

Sodium salicylate has antipyretic and analgesic actions. When

given by mouth, it is absorbed readily and rapidly throughout the GI tract and rapidly excreted; frequent doses are therefore required to maintain a satisfactory concentration in the blood. The usual dose is 0.6 to 2 g (British Pharmaceutical Codex, 1973).

Sodium salicylate is a gastric irritant and sodium bicarbonate is often given with it to reduce this effect; however, the bicarbonate also increases the rate of excretion and thus lowers the concentration of salicylate in the blood to less effective levels.

The pharmacology of salicylates and related compounds has been reviewed extensively by Smith (1960). Salicylates in general, exert their antipyretic action in febrile patients by causing dilatation of the skin vessels and some perspiration and the increased loss of heat results in a fall in body temperature. The principal use of sodium salicylate is in the treatment of acute rheumatic fever; a dosage of 1.3 g may be given by mouth every 2 hr.,or 2 g every 3 hr., until the temperature is reduced. For acute rheumatism, it is given in a daily dosage of 5 to 10 g in divided doses (British Pharmaceutical Codex, 1973).

Salicylate must be used with care in patients with acute renal disease. The prothrombin time may be prolonged after repeated large doses.

(c) Absorption, distribution and elimination of salicylate

Although the small intestine is now regarded as the major site of absorption of most drugs from the GI tract, because of the large area of the intestinal mucosa (see Part 1.4.3a in Section 1), several reports suggested that absorption from the stomach may account for an appreciable fraction of the total dose absorbed in the case of salicylates, aspirin and SA (Levy, 1961; Levy et al, 1961; Truitt and Morgan, 1960 and 1964; Rowland et al, 1967; Saunders, 1974a; Nayak

and Benet, 1974).

The rate of absorption is limited by the low solubility of the acids at the gastric pH values (Saunders, 1974a). Earlier indication of the gastric absorption of SA was provided by Carnot et al (1932), who reached the conclusion that "absorption of sodium salicylate was positive when the stomach contents were acid and negative when the gastric contents were neutral or alkaline".The absorption in the acid stomach was so rapid that salicylate was detected in serum from the third minute after the salt solution was introduced into the dog stomach ligated at both ends.

Rowland et al (1972) reported that the absorption of salicylate appears to follow first order kinetics. The general picture, however, is that the salicylate and related compounds penetrate the intestinal blood barrier by passive diffusion of the un-ionized molecule across a membrane having lipid characteristics. In absorption from the stomach the low pH of the contents means that an anionic drug is almost completely un-ionized whereas at the average plasma pH (7.4) most anionic drugs are highly dissociated. There is,therefore,a concentration gradient of un-ionized form which provides a gradient for diffusion (Saunders, 1974a).

There appear to be three principal variables, among others, which can affect the results of comparative salicylate absorption studies from orally administered dosage forms; the physical dosage form, intragastric pH and gastric emptying time (Truitt and Morgan, 1964; Levy et al, 1961).

The initial rate of salicylate absorption is proportional to the dissolution rate of the drug in its particular dosage form. Dissolution is, in fact, the rate controlling process in aspirin absorption (Levy

et al, 1961). Absorption is rapid when salicylate is administered in solution; maximum plasma aspirin levels are achieved within 15-25 minutes after oral dosing. The absorption rate from solution varies among individuals and appears to be influenced by physiological conditions in the GI tract (Rowland et al, 1967). The absorption rates were similar when aspirin was administered as a solution of sodium and choline salts, but these rates were significantly higher than that obtained with aspirin tablets (Levy et al, 1961). This result was attributed to the fact that both sodium and choline salicylates being essentially fully ionised salts of the same weak acid, revert to un-ionised SA in acidic gastric fluid. It is the un-ionised SA, rather than its salts, which is absorbed by the process of passive diffusion from the GI tract. These observations and suggestions are in agreement with the earlier conclusion of Carnot et al (1932), who used sodium salicylate. Levy (1961) studied the absorption rates of a number of commercial aspirin preparations in vivo and found that the relative absorption rates were proportional to in vitro dissolution rates of aspirin from the preparations. Absorption of sodium salicylate and aspirin from solution is much more rapid than from solid tablets. However, antacids lower the rate of absorption from solution by reducing the amount of undissociated aspirin present but increase the rate of absorption from solid tablets, because their effect in increasing the rate of dissolution of solid aspirin outweighs the effect due to decrease of un-ionised aspirin (Truitt and Morgan, 1964).

Increase in the gastric emptying rate (GER) leads to enhancement in the absorption rate of salicylate as a result of rapid appearance of the drug to the major absorption site, i.e. the small

intestine (Lolli and Smith, 1946; Sleight, 1960; Moore et al, 1960) (see Part 1.4.3a, Section 1). However, Cook and Hunt (1970) pointed out the importance of GER on the absorption of aspirin. They found that, after 10 min., there was at least 10 times less absorbed when aspirin was administered in a buffered solution compared with an unbuffered one. It was suggested that the reason for this was that the higher the pH the faster is the gastric emptying and hence the shorter the time for absorption. According to the pH partition theory (see Part 1.3.1, Section 1), for acidic drugs, like salicylates, the stomach is the optimal site of absorption. Thus, the longer the gastric residence time the higher will be the absorption. The bioavailability of salicylate and related compounds from different dosage forms has been extensively reviewed by Saunders (1974a) and Mayersohn et al (1977).

Since the metabolite of aspirin, i.e. SA, is pharmacologically active, its distribution in the body is important and in bioavailability studies on dosage forms containing salicylate derivatives SA levels in the body must be taken into account (Mayersohn et al,1977). Once the salicylate derivative is converted to SA, it follows the metabolic pathway of the latter. The metabolic products of SA are indicated by Florey (1979) and major ones are salicyluric acid and salicyl glucuronide, which are formed by conjugation with glycine and glucuronic acid respectively.

The elimination of salicylate occurs mainly by the parallel processes of renal excretion of unchanged SA and metabolism. However, the major metabolic pathways are easily saturated in the usual dosage range that is used for the treatment of inflammation (Levy et al, 1972). Thus, metabolic conversion of salicylate becomes less efficient as the dose of salicylate increases. Therefore, since the

fraction of the dose of a salicylate, e.g. aspirin, that is excreted as unchanged salicylate increases with dose (Mayersohn et al, 1977), the proportional contribution of metabolism to the overall elimination process is reduced for large doses. Consequently, elimination of salicylate appears to be a first-order process because the contribution of the zero-order process is too small to be noticeable, except during the terminal phase of salicylate elimination (Levy, 1965).

With the pH-partition theory in mind (see Part 1.3.1, Section 1) and the fact that good gastric absorption of salicylate occurs it is obvious that GER plays a vital role in salicylate absorption. Since the GER is known to be delayed by lipid (see Chapter 2, Section 1), it was decided to study the bioavailability of sodium salicylate administered in oily vehicle. Sodium salicylate was used as a model compound which possesses a low solubility in the oil but a high solubility in water. This chapter is limited to a consideration of a simple oily suspension of sodium salicylate in order to study the effect of the oil alone on the bioavailability of sodium salicylate. In the subsequent chapter the effects of pharmaceutical additives on the bioavailability of sodium salicylate administered in oily suspension are considered.

1.2 Experimental

(a) Materials

Sodium salicylate and the reagents used in the determination of blood salicylate concentrations, i.e. Analar ferric nitrate and mercuric chloride, were obtained from B.D.H. Chemicals Ltd. and the Fractionated Coconut Oil B.P.C. 1968 was obtained from Alembic Products Ltd.

(b) Methods

Sodium salicylate was sieved and the 100/120 portion (mesh size 125-150 μm) was used to prepare the dosage forms as either 4% w/v solutions in distilled water or 4% w/v suspensions in Fractionated Coconut Oil. The suspension was homogenised for one minute using an Ultra-Turrax mixer at a fixed speed. Both dosage forms were stored overnight. On the following morning the suspension was stirred vigorously before the required dose volume was withdrawn.

Adult male New Zealand white rabbits, weighing 3.94-4.69 kg and fed with a standard diet, were used in this study. Doses of 120 mg/kg body weight (equivalent to a dose volume of 3 cm^3/kg body weight) of sodium salicylate were administered as either of the above mentioned dosage forms by means of a catheter and syringe directly into the stomachs of rabbits that had been fasted for 20 hr. The catheter was flushed out with 1/3rd of the dose volume of the appropriate vehicle, i.e. water or oil, before removal from the rabbit. The dose was based on the work of Lessel and Cliffe (1964) and a 2-way cross-over design utilising 8 rabbits was used for the study. Fasting was continued during the first 9 hr of each experiment. Blood samples (0.7 cm^3) were taken from the marginal ear vein, using a 1 cm^3 heparinised syringe, immediately before administration of the drug and at specified times during the 24 hr post-administration period. The samples were placed in heparinised tubes and stored in a refrigerator until the next day when they were assayed. The total blood salicylate content in each sample was determined by Trinder's method (Trinder, 1954), which was carried out as follows:
0.7 cm^3 of blood was placed in a cylindrical centrifuge tube and

3.5 cm³ of Trinder's colour reagent* were added. The tube was shaken during the addition and shaking was continued for a few seconds to ensure that the precipitated protein was finely dispersed. After centrifugation at 2000 g for 5 minutes the supernatant was withdrawn gently using a Pasteur pipette and its absorbance was determined at 540 nm in a Unicam SP 500 spectrophotometer against a blank solution containing 0.7 cm³ of water and 3.5 cm³ of Trinder's reagent. The concentration of salicylate in each sample was calculated from a calibration curve obtained by measuring the absorbances of a series of known concentrations of salicylate in rabbit blood, after treatment with Trinder's colour reagent. The Beer-Lambert law was obeyed over the concentration range used for the calibration curve. The concentrationsand absorbances of these solutions are given in Table 1.1.

Table 1.1. Data for calibration curve of sodium salicylate in rabbit blood at 540 nm

Concentration mg/100 cm³	Absorbance
10	0.18
20	0.36
30	0.535
40	0.725
50	0.90

*Trinder's colour reagent:

40 g of A.R. mercuric chloride were dissolved in 850 cm³ of distilled water with the aid of heat. The solution was cooled and 120 cm of HCl (1 mole/dm³) and 40 g of ferric nitrate ($Fe(NO_3)_3$, $9H_2O$) A.R. were added. When all the ferric nitrate had dissolved the volume of the solution was made up to 1000 cm³ with distilled water. This solution is stable for long periods.

The regression coefficient (b) of this curve was calculated and the concentrations of salicylate in the blood samples were obtained using the following equations,

$$Y - \bar{Y} = b\ (X - \bar{X}) \qquad \text{Eq. 1.1}$$

or

$$X = \frac{(Y - \bar{Y}) + b\ \bar{X}}{b} \qquad \text{Eq. 1.2}$$

where X and Y are the blood salicylate concentration and absorbance, respectively and $\bar{X}$ and $\bar{Y}$ are the mean values. Inserting the values for $\bar{X}$, $\bar{Y}$ and b into Eq. 1.2 gives Eq. 1.3.

$$X = \frac{Y + 0.0015}{0.01805} \qquad \text{Eq. 1.3}$$

All the studies were initiated at the same time of day in order to eliminate possible circadian variation. The design of this study based on a 2-way cross-over design utilising 8 rabbits is described in Table 1.2(Wagner, 1975b).

Table 1.2 2-way cross-over design

Group	Subjects per group, number of rabbits	Time Periods I	II
1	1 - 4	A	B
2	5 - 8	B	A

The formulation (dosage form) contained sodium salicylate 4% w/v (A)suspended in Fractionated Coconut Oil and(B)dissolved in distilled water.

1.3 Results

The mean concentrations of salicylate in the blood samples that were obtained at various times after oral administration of sodium salicylate are given in Table 1.3 and plots of these data are shown

in Fig.1.1 for each formulation. The values of the three commonly used bioavailability parameters, i.e. area under the blood concentration versus time curve (AUC), peak blood concentration (PC) and the time at which this concentration is reached (PT), were obtained from plots of the blood concentration versus time curves for each rabbit and each formulation and are given in Table 1.4. The AUCs for the 0-24 hr. post-administration period obtained from individual experiments were calculated by the trapezoidal method (Notari, 1980a).Thus, the $(AUC_o)^{24}$ were calculated from the following equation:

$$AUC_o^{24} = \frac{(C_0 + C_{0.5})\ 0.5}{2} + \frac{(C_{0.5} + C_1)0.5}{2} + \frac{(C_1 + C_2)}{2}$$

$$+ \frac{(C_2 + C_3)}{2} + \frac{(C_3 + C_4)}{2} + \frac{(C_4 + C_5)}{2} + \frac{(C_5 + C_6)}{2}$$

$$+ (C_6 + C_9)3 + (C_9 + C_{24})15$$

where C represents the concentration of drug in the blood at the time in hours denoted by its subscript.

Table 1.3 Mean blood concentrations (mg/100 cm^3) in rabbits following administration as a single dose (120 mg/kg body weight) of sodium salicylate in two formulations. Each value is the average of 8 experiments.

formulation (a) \ Time(hr)	½	1	2	3	4	5	6	9	24
A	19.5	23.1	24.2	24.0	23.6	22.3	21.1	19.5	9.6
B	24.8	28.2	24.8	22.5	20.8	19.2	18.3	14.6	2.8

(a) Key A = oily suspension

B = aqueous solution

Fig. 1.1 Mean blood concentrations versus time after administration of 4% w/v sodium salicylate (☼) suspended in FCO and (✸) dissolved in distilled water. Each curve is the average of results obtained in 8 rabbits.

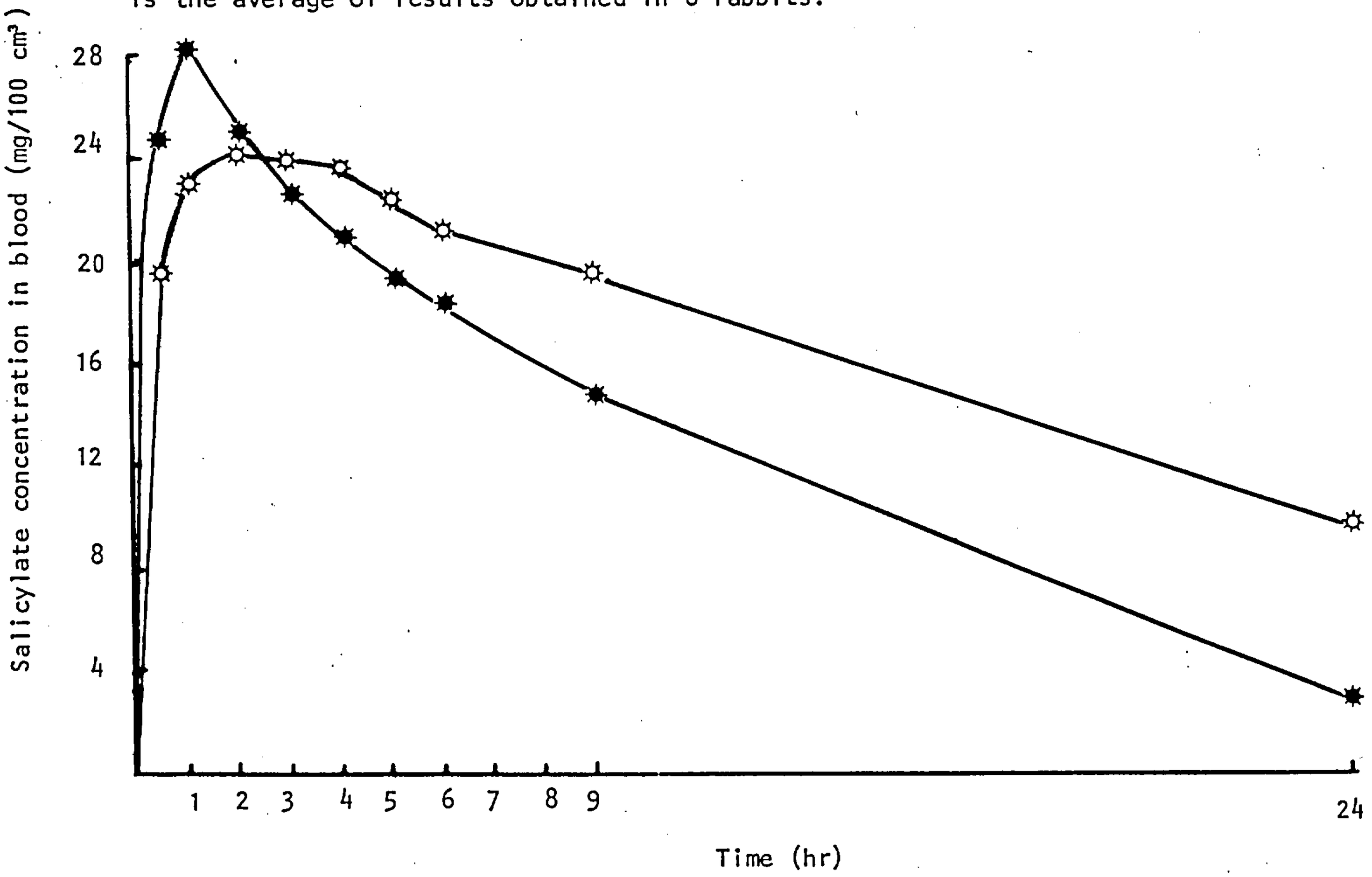


Table 1.4 Bioavailability parameters obtained following oral administration of sodium salicylate in aqueous solutions and oily suspensions to rabbits.

Rabbit No.	Peak Concn. (mg/100 cm³)		Peak Time (hr)		AUC_0^{24} (mg hr/100 cm³)	
	Soln.	Suspn.	Soln.	Suspn.	Soln.	Suspn.
1	27.7	27.6	1	1	425.1	422.2
2	29.9	27.2	1	2	416.8	426.8
3	35.6	29.8	1	2	306.8	398.5
4	30.4	21.9	0.5	4	173.7	459.1
5	23.9	24.4	1	2	252.1	362.5
6	21.8	24.9	2	2	221.5	453.6
7	35.1	22.5	1	4	374.0	413.4
8	25.4	20.9	3	4	311.7	350.7
Mean	28.7	24.9	1.3	2.6	310.2	410.9
	n.s.d. ($p > 0.05$)		sig. diff. ($p < 0.05$)		sig. diff. ($p < 0.05$)	

The statistical analysis of the bioavailability parameters was carried out according to the method given by Wagner (1975). As an example the details of the analysis of the peak times (hr) are given below.

Treatment

Group	Subject	A	B	Subject total
	1	1	1	2
	2	2 Week I	1 Week II	3
1	3	2 Sum = 9	1 Sum = 3.5	3
	4	4	0.5	4.5
	5	2	1	3
	6	2 Week II	2 Week I	4
2	7	4 Sum = 12	1 Sum = 7	5
	8	4	3	7
		T_1 = 21	T_2 = 10.5	Sum = N = 31.5
		Ave = 2.6	Ave = 1.3	

W1 = 9 + 7 = 16

W2 = 12 + 3.5 = 15.5

G1 = 9 + 3.5 = 12.5

G2 = 12 + 7 = 19

Correction factor = $\frac{(31.5)^2}{16}$

C.F. = 62.02

Sum of Squares

Group	Subject	A	B
	1	1	1
1	2	4	1
		Sum = 25	Sum = 3.25
	3	4	1
	4	16	0.25
	5	4	1
2	6	4 Sum = 40	4 Sum = 15
	7	16	1
	8	16	9
		Sum = 65 +	18.25 = 83.25 = Total

uncorrected sum of square.

Sum of squares total, $SS_{total} = 83.25 - 62.02 = 21.23$

$$SS_{treatments} = \frac{(21)^2 + (10.5)^2}{8} - 62.02 = 6.89$$

$$SS_{subjects} = \frac{(2)^2 + (3)^2 + (3)^2 + (4.5)^2 + (3)^2 + (4)^2 + (5)^2 + (7)^2}{2} - 62.02$$

$$= 8.61$$

$$SS_{weeks} = \frac{(16)^2 + (15.5)^2}{8} - 62.02 = 0.01$$

$$SS_{residual} = SS_{total} - (SS_{treatment} + SS_{subjects} + SS_{weeks})$$

$$SS_{residual} = 21.23 - (6.89 + 8.61 + 0.01) = 5.72$$

$$SS_{groups} = \frac{(12.5)^2 + (19)^2}{8} - 62.02 = 2.64$$

$$SS_{subjects/groups} = SS_{subjects} - SS_{groups} = 8.61 - 2.64 = 5.97$$

Table 1.5 ANOVA[a] of PT of salicylate in blood following administration of two formulations of sodium salicylate

Source of variation	df[b]	SS	MS[c]	F[d]
Total	15	21.23	1.42	1.5
Subjects:	7	8.61	1.23	1.30[e]
Groups	1	2.64	2.64	2.78
Subj/Group	6	5.97	1.00	1.05
Weeks	1	0.01	0.01	0.01
Treatments	1	6.89	6.89	7.25
Residual (Error)	6	5.72	0.95	

The critical value of $F_{1,6}$ at 5% probability level is 5.99. Thus, there is a significant difference between the mean values of the peak time because the critical value is less than the $F_{1,6}$ of the treatment, i.e. 7.25, given in Table 1.5.

(a) ANOVA = Analysis of variance, (b) d.f. = degree of freedom,

(c) Mean of squares, $MS = \frac{SS}{d.f.}$, (d) $F = \frac{\text{MS of treatment, subject etc.}}{\text{MS error.}}$

(e) The F ratio for subjects (rabbits) of 1.3, ($v_1 = 7$, $v_2 = 6$) where $F_{7,6}$ at 5% probability level is 4.21, is not significant thus indicating homogeneity in the animals. However, in some instances in this thesis similar F values are significant, but because of the experimental design this in no way invalidates the conclusions drawn about the significance of the treatments.

Similar statistical analyses were carried out for the AUC_o^{24} and PC values and the results are summarised in Table 1.6 together with those for PT.

Table 1.6 Mean values of peak time (PT), peak concentrations (PC) and the area under the curve (AUC_o^{24}) after administration of sodium salicylate in two formulations in a 2-way cross-over design to 8 rabbits.

	Formulation	
	A	B
PT (hr)	2.6	1.3
PC (mg/100 cm^3)	24.9	28.7
AUC_o^{24} (mg hr/100 cm^3)	410.9	310.2

Any two means not underscored by the same line are significantly different ($p<0.05$). Any two means underscored by the same line are not significantly different.

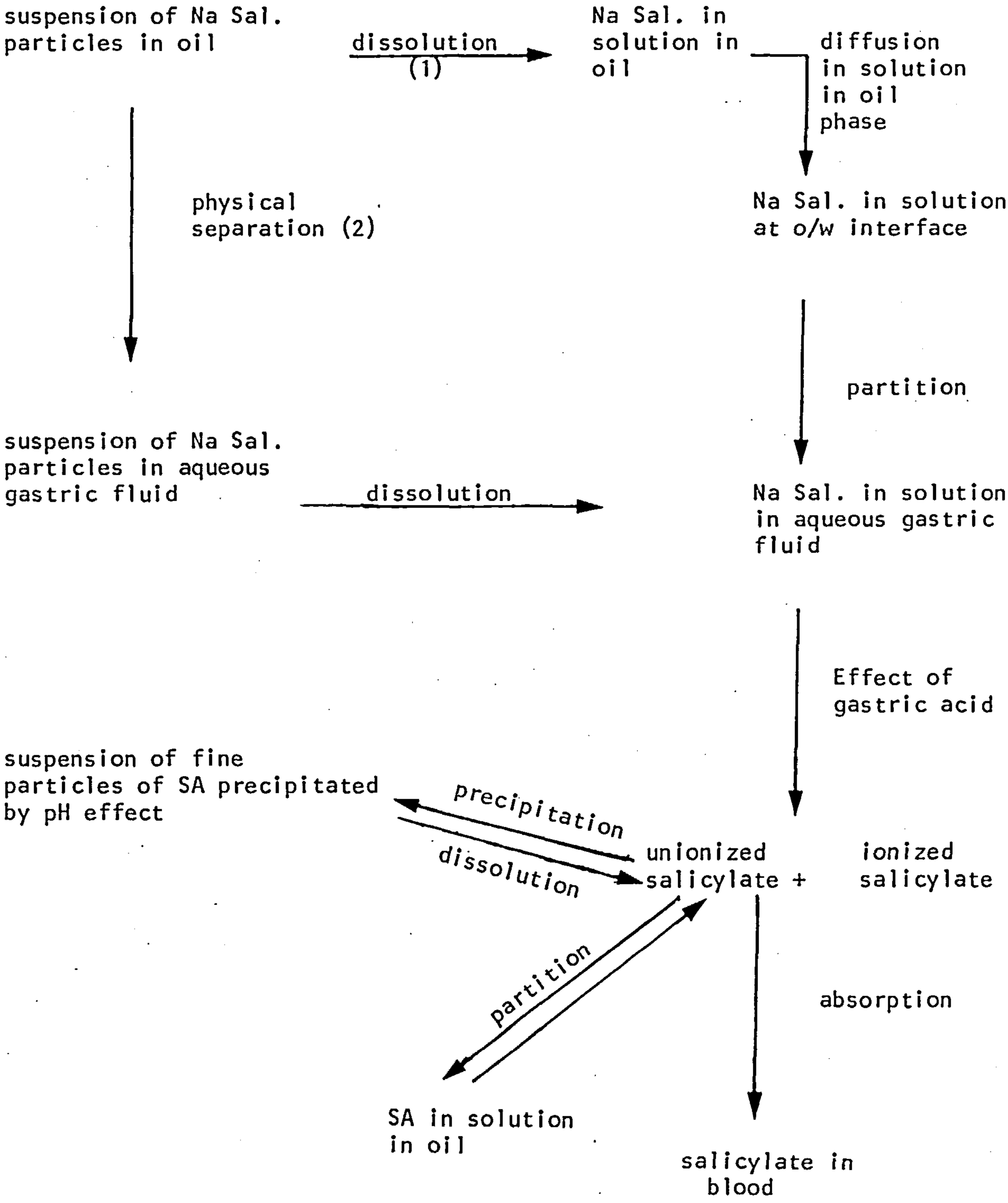
1.4 Discussion

Examination of the curves in Fig. 1.1 and the mean PT values given in Table 1.4 suggests that a statistically significant delay occurred in the attainment of the peak blood concentration following administration of the oily suspension when compared with the aqueous solution. It should be pointed out that the precision with which the PT values can be derived from those plots relating to the oily suspensions is lower than that obtainable for the aqueous solutions, because the latter produced relatively sharp peaks in the blood concentration versus time profiles whereas the oily suspensions gave rise to broader peaks. This reservation should be borne in mind

if the above mentioned difference in PT values is interpreted as an indication that the rate of bioavailability of salicylate from the oily suspension is reduced in comparison to that from the aqueous solution. However, although the difference between the mean PC values shown in Table 1.4 is not statistically significant the trend towards a lower value for the oily suspension is in keeping with such interpretation. Furthermore, it is to be expected that the rate of availability will be faster in the case of the aqueous solution since greater initial concentrations of salicylate are more likely to be produced in the aqueous GI fluids when this formulation is administered. It should be borne in mind that the low gastric pH may cause precipitation of salicylic acid. In the event of such precipitation the solid particles that are produced are considered to be sufficiently small to provide relatively rapid rates of subsequent dissolution (Munzel, 1971). Enhancement of rate of absorption of acidic drugs by the administration of water soluble salts of the parent acids has been demonstrated for tolbutamide (Nelson et al, 1962), p-aminosalicylic acid (Wan et al, 1974) and aspirin (Levy, 1961; Leonards, 1962).

When an oily suspension of sodium salicylate is administered then additional processes must be included in the bioavailability pathway. For example, the drug must either first dissolve in the oil and then partition into the aqueous fluids of the GI tract or transference of the undissolved drug particles from oil to aqueous phase followed by dissolution must occur. The situation will be further complicated by the ease with which SA, formed by hydrolysis of the sodium salt in the gastric acid, repartitions into the FCO. The following scheme illustrates these possibilities.

Scheme 1. Stages in the bioavailability pathway for sodium salicylate administered orally as a suspension in an oily vehicle



The effect of conversion of the sodium salt to salicylic acid in the acidic aqueous phase on the distribution of salicylate in such a system is indicated by the relatively high apparent partition coefficient (38.6) of sodium salicylate between FCO and 0.1 mole/dm^3 HCl at 37°C. This coefficent is contrary to that expected on the basis of the solubilities of sodium salicylate in the oil (16.85 mg/100 cm^3) and in 0.1 mole/dm^3 HCl (306 mg/100 cm^3) (see Chapter 2, Section 4). The pathway (2) in Scheme 1 is more likely than pathway (1). This is probably related to the ease of the physical separation of solid drug particles from the oil followed by the rapid dissolution process. This probability is supported by _in vivo_ and _in vitro_ studies on highly water soluble drugs administered as suspensions in a non-aqueous vehicles,e.g. fatty suppository bases and liquid paraffin, e.g. sodium salicylate in fatty suppository bases (Schoonen et al, 1976, 1979, and 1980), phenobarbitone sodium and sodium chloride in fatty suppository bases (Rutten-Kingma et al, 1979a and c) and sodium chloride in liquid paraffin (Crommelin and de Blaey, 1980a).

Absorption of drugs administered as solutions in lipids is considered to involve liberation of the drug from the vehicle into the aqueous luminal fluid, followed by passage through the GI wall. Armstrong et al (1979) determined _in vitro_ rates of release and distribution coefficients of a range of benzoic and phenylacetic acids between either isopropyl myristate or octanol and water, and compared them with the bioavailabilities in rat. Absorption _in vivo_ followed an inverse rank order to lipid solubility, but was related to the _in vitro_ solvent-water transfer rate constant, rather than the distribution coefficient. The inference was, therefore, that availability depended on the amount (Kakemi et al, 1972a) or concentration (Grisafe and

Hayton, 1978) of the drug in solution in the GI fluids, which in turn was dependent on the rate of supply from the oily phase. In other words, in situations where this is slow in comparison with the rapid absorption from the aqueous phase, as in the case of salicylate, lipid and aqueous phases *in vivo* may not be in equilibrium, and the transfer from the oil to water becomes the rate-determining process (Armstrong and James, 1980). Furthermore, Grisafe and Hayton (1978) have shown that the absorption rate of dissolved griseofulvin from the rat intestine decreases in the presence of triglyceride digestion products because the drug concentrates in the micellar and oil phases and negligible absorption occurs from these phases. Ogata and Fung (1980) reported that nitroglycerin absorption appeared slower from a sesame oil emulsion vehicle compared with aqueous solution. The authors suggested that, "because of high partition coefficient between the oil and water at 37°C (76), the organic nitrate resides principally in the oily internal phase of the emulsion vehicle, thus possibly delaying its release into intestinal fluids".

Therefore, it is likely that the salicylic acid formed in the acidic medium of the stomach resides principally in the oily phase in the GI tract and the oil will probably provide a reservoir for the uptake of the salicylic acid and so reduce the amount of drug initially available for absorption by controlling its release to the GI fluids (Bloedow and Hayton, 1976; Ogata and Fung, 1980; Armstrong and James, 1980). Since the initial absorption of salicylate occurs from the stomach, and since the rate of this absorption is proportional to the amount of salicylate dissolved in the gastric fluids, the *in vivo* dissolution rate of salicylate in the stomach would be reflected by the initial absorption rate (Levy, 1961). Indeed, this appears to be the

case in this study, since the results of salicylate absorption in the initial phase parallels the in vitro release of the drug at 37°C using 0.1 mole/dm³ HCl as the dissolution medium (see Chapter 1, Section 4). Furthermore, similar correlations between in vivo and in vitro results were not obtained when using drugs with very small oil/0.1 mole/dm³ HCl partition coefficients, e.g. ampicillin trihydrate and nitrofurantoin (see Chapter 1, Section 4). These results suggest that the viscosity of the oil plays an insignificant role in delaying the rate of absorption but its role as a reservoir for lipid soluble drugs is important. Thus, the more lipophilic the drug the more reluctant it will be to migrate to the GI fluids (Armstrong and James, 1980).

The rate of absorption of salicylate will also be affected by the fact that oil delays the emptying rate of the stomach (see Chapter 2, Section 1), and consequently decreases the rate of appearance of the drug in the small intestine. This latter site is regarded normally as the optimum site of absorption for most drugs; even if the drugs are readily absorbed from the stomach such as aspirin and related drugs (Levy, 1961) and even if the drug is ionised in the intestine and non-ionised in the stomach (Benet, 1973). It has been recognised that acceleration of gastric emptying can increase the rate of aspirin absorption (Lolli and Smith,1946; Sleight, 1960). Moore et al (1960) found that faster stomach emptying increased the toxicity and shortened the onset of action of sodium salicylate.

Although the rate of absorption is reduced by the administration of sodium salicylate as a suspension in oil the AUC values given in Table 1.4 show that the amount of salicylate absorbed is significantly greater than in the case of the aqueous solution. A variety of factors could be possible for this enhancement, e.g. (i) delay in GER caused by

the oil; (ii) decrease in gastric secretion and hence increase in gastric pH; (iii) enhancement of the uptake of the drug by the stimulation of the lymph flow; (iv) formation of mixed micelles; (v) viscosity of the oil.

(i) Although salicylates are mainly absorbed under normal conditions from the small intestine appreciable gastric absorption of aspirin and salicylate has been reported (Truitt and Morgan, 1960 and 1964; Nayak and Benet, 1974; Saunders, 1974a). It appears, therefore, that an increase in gastric residence time might lead to an increase in the contribution that such absorption makes to the overall extent of absorption. In addition, the slower release of drug from the stomach may improve the efficiency of absorption from the intestine or allow a longer period for drug dissolution to occur before transfer into the intestine. It is suggested, therefore, that the increase in extent of absorption of salicylate, that is obtained when sodium salicylate is administered in an oily suspension rather than as an aqueous solution, may be ascribed to the reduction in stomach emptying rate that is caused by the presence of oil (see Chapter 2, Section 1). Various examples of the enhancement of absorption of weak acidic drugs that can be ascribed to delays in the GER have been given in Part 1.4.1a, Section 1.

(ii) Intragastric dissolution would also be enhanced by any increase in stomach pH that results from the reduction in gastric secretion caused by the presence of oil in the duodenum (Johnson and Grossman, 1969; Christiansen et al, 1976). Changes in gastric pH, brought about by the administration of antacids (Hurwitz, 1971) or by achlorhydria (Pottage et al, 1974), have been shown to have significant effects on the absorption of weak acids and these effects are ascribed to the relationship between the solubilities of these compounds and pH.

Factors that affect dissolution rate may be of significance in the case of poorly soluble drugs administered as solids. However, they offer less likely explanations of the present results, where the lower extent of bioavailability is exhibited by an aqueous solution, unless the effects of precipitation of salicylic acid in the stomach from solution of sodium salicylate have a greater importance than realised hitherto.

(ii) Bloedow and Hayton (1976) suggested that the absorption of lipophilic drugs might be enhanced by the coadministration of lipids due to absorption of these drugs via the lymph by incorporation in the chylomicrons. The existence of a special transport mechanism, by which drug and oil are transported together into the lymphatic system when injected into the stomach wall, has been reported (see Part 1.4.3c, Section 1).

Fatty acids containing 14 or more carbon atoms are considered to be taken up in the lymph through chylomicron formation and those containing 8-12 carbon atoms enter the systemic circulation through the portal vein (Bloom et al, 1951). It has been suggested that lipophilic drugs follow one or both of these routes, depending on the nature of the drug (Bloedow, 1974) and the nature of the oil (Bloedow and Hayton, 1976). It is unlikely, therefore, that salicylic acid would be absorbed through the lymphatic route in this study since FCO possesses hydrocarbon chains with 8-10 carbon atoms and will consequently be absorbed mainly through the portal vein. Supporting evidence for this suggestion is provided by Palin et al (1980) who found that the absorption of very lipid soluble DDT was enhanced when administered in fractionated coconut and arachis oils and suggested that this enhancement was due to the effect of the oils on the total gut

transit rate in spite of the fact that DDT is preferentially absorbed via the lymphatic route. Furthermore, De Marco and Levine(1969) reported that the stimulation of lymph following the coadministration of tripalmitin would have little effect on a well absorbed drug. Its effect would be significant with the agents that are only slightly absorbed at best. Therefore, the effects of enhanced lymph flow are unlikely to provide an explanation of the results obtained in this study.

(iv) Enhancement of the absorption of a variety of non-acidic drugs, e.g. heparin and streptomycin, by administration in oily formulations has been reported recently. The formation of mixed micelles, composed of lipids and bile salts, and their effects on the permeability of the absorbing membrane have been suggested as a possible mechanism (Muranishi et al, 1977 and 1979; Taniguchi et al, 1980; Muranushi et al, 1980a and b). This mechanism offers a less likely explanation of the present results since the delay in GER keeps the salicylate in the stomach for a longer period of time and according to the pH-partition theory SA is well absorbed from this site. However, the effects of mixed micelle formation may become significant after the drug is transferred to the small intestine where the mixed micelles are formed.

(v) Although the viscosity of the oil is higher than water (17.5 vs 0.695, mN s m^{-2} respectively) (see Section 2), this appears to have a negligible influence on the extent of absorption because further increase in the viscosity by incorporation of 1% w/v and 4% w/v aluminium stearate (see next Chapter) had no significant effect on the extent of absorption of salicylate. It is suggested that the delaying effect of the oil on GER predominates and masks the effect of viscosity.

The conclusions made in the previous paragraphs concerning the effect of oil on the extent of absorption of salicylate are based on the assumption that the AUC values can be used as estimates of the relative amounts of drug absorbed from the two different formulations. This assumption is only correct if the shape of the blood level curve can be described in terms of linear pharmacokinetics. The above conclusions can therefore be criticised because, as pointed out in the introduction to this chapter, it is known that a capacity limited metabolic pathway is involved in the elimination of salicylates from man (Levy, 1965; Levy et al,1972). However, many bioavailability studies on salicylate dosage forms have ignored the non-linearity of the kinetics (e.g. Truitt and Morgan, 1960 and 1964; Nayak and Benet, 1974; Wan et al, 1974; Orozco-Alcala and Baum, 1979 and Barzegar-Jalali and Richards 1979b), including in vivo/in vitro correlation studies, such as those of Levy (1961), Levy et al (1961) and Nayak and Benet (1974), which are among the best known correlations of this type. Furthermore, although Mayersohn et al (1977) reported that AUC values could be used to provide valid assessments of the extent of salicylate absorption in man provided a dose of 500 mg was not exceeded, Nayak et al (1977) used single doses of 650 mg and obtained a reasonable fit of their experimental data to a linear one compartment model in spite of the fact that they were aware of a capacity limited metabolic pathway.

One test of the non-linearity of pharmacokinetic data as indicated by Wagner (1975a) involves the administration of a drug at two or more dose levels. Each concentration on the respective blood concentration versus time curves is then divided by the dose or normalised dose and these ratios are replotted against time. If

the curves are not superimposable or nearly so then one may expect some type of non-linearity. In addition, the AUC_o^{∞} values for the initial blood level curves should show a similar ratio to the doses if linear pharmacokinetics are applicable. This approach was tried using the data presented in this chapter for the suspension of sodium salicylate in FCO that was administered at a dose of 120 mg/kg and data presented in the next chapter for the same formulation (Formula A) but administered at a dose of 60 mg/kg. The normalised blood level curves for these two sets of data are approximately superimposable from t = 0 to t = 9 hr, which is the last sampling time used in the experiment described in the next chapter. In addition, estimated values of AUC_o^{∞} were obtained using Eq. 1.4,

$$AUC_o^{\infty} = AUC_o^{t(last)} + \frac{c_{t(last)}}{k} \qquad \text{Eq. 1.4}$$

where $AUC_o^{t(last)}$ is the area under the blood level curve from zero time until the last sampling time (t(last)) as determined using the trapezoidal rule, $^{c}t(last)$ is the concentration of salicylate in the blood at the last sampling time as estimated from the linear portion of a semi-log plot of concentration versus time and k is the mean slope of the linear portions of the individual semi-log plots of the data presented in this chapter for the suspension of sodium salicylate in FCO.

The values of AUC_o^{∞} obtained for the two dose levels were 582 and 262 mg hr/100 cm^3 respectively. Their ratio is 2.2, which is close to the ratio of the two doses, i.e.,2.0.

The closeness of the normalised blood level curves and the agreement between the AUC_o^{∞} ratio and dose ratio suggest that the system can be described approximately by linear pharmacokinetics.

In view of all the above comments it seems reasonable to suggest that the possible criticism of the use of AUC values as indicators of the relative extents of salicylate absorption from the different formulations is not very severe.

In conclusion, it is suggested that the enhancement in the extent of salicylate absorption obtained in this study can be attributed largely to the effect of oils on total gut transit time. In addition, it is suggested that the reduction in the rate of absorption is due partly to the action of the oil as a reservoir that controls the release of salicylic acid and partly to the delay in the appearance of salicylate in the small intestine because of the decrease in the GER.

If oily suspensions of sodium salicylate do provide a means of reducing the rate of salicylate absorption whilst enhancing the extent of absorption then such formulations may be of value in the treatment of chronic rheumatism by allowing a reduction in either the dose or its frequency of administration. However, it should be pointed out that the volume of oil used in the present studies is relatively high when compared with the dose volumes that would be used normally in humans and Yamahira et al (1978) have demonstrated that dose volume is an important factor in determining the effects of lipids on the bioavailability of an anti-inflammatory agent in rats. Thus, the results obtained in the present study may have more significance in relation to the effects of fatty meals on drug bioavailability.

CHAPTER 2

BIOAVAILABILITY STUDIES ON DIFFERENT OILY FORMULATIONS OF SODIUM SALICYLATE IN THE RABBIT

2.1 Introduction

In the previous chapter, the bioavailability of sodium salicylate from an oily suspension was compared with that from an aqueous solution. However, rarely is a drug alone or in a simple vehicle used in therapy. It is usually administered in a pharmaceutical dosage form which contains, besides the active ingredient, the necessary pharmaceutical adjuvants or excipients, such as suspending agents, surface active agents(surfactants), colouring and sweetening agents etc.

There is now considerable evidence that the bioavailability of a drug can be markedly affected by the physical state of the drug and the dosage form in which it is administered. Formulation factors may affect the onset, intensity and duration of patient response, and also the incidence and intensity of side effects, most frequently due to differences in the rate at which the active ingredient or ingredients become available for absorption. This availability for absorption may be a function of formulation variables (Hirst and Kaye, 1971), a dissolution rate limited absorption process (Yamamoto et al, 1974), the effect of oil (Carrigan and Bates, 1973; Bates and Sequeria, 1975; Bates et al, 1977; Chakrabarti and Belpaire, 1978; Ogata and Fung, 1980), effect of osmatic pressure (Kato et al, 1969; Marvola et al, 1979b), or effect of viscosity (Levy and Jusko, 1965; Marvola et al, 1979a; Barzegar-Jalali and Richards, 1979b and 1980; Soci and Parrott, 1980).

Hirst(1976) in his review "Formulation and bioavailability", summarised the effect of formulation as follows:

"Any reduction in the bioavailability of a drug is, in effect, a reduction in the dose of drug administered. Different formulations of the same drug cannot be assumed to be therapeutically equivalent simply because they contain the same amount of drug and comply with official standards. Any change, however small apparently, cannot be assumed to have no effect on the bioavailability of the drug. Many minor changes to drug formulations and methods of manufacture have in some instances had a disastrous effect on the bioavailability of the drugs included in the preparations".

Non-aqueous vehicles for oral pharmaceutical suspensions of water degradable physiologically active agents are the subjects of patents (Stephens and Su, 1975; Lin and Pramoda, 1978) (see Chapter 4, Section 1). Although the latterpatent infers that the bioavailabilities of drugs suspended in the oily vehicle are equal to those of aqueous suspension, only data relating to the bioavailability of amoxicillin are presented. No further studies were conducted to evaluate the effect of the different additives on the bioavailability of drugs. In addition, no mention of the effect of the oily vehicle on bioavailability was made in the patent of Stephens and Su (1975).

Therefore, it was the purpose of this study to investigate the effect of the different pharmaceutical additives used in the above mentioned patents, individually and in combination, on the bioavailability of sodium salicylate in the rabbit.

2.2 Experimental

a) Materials

Details of the sources of the materials and methods of preparation of the oily vehicles are given in Section 2 and Chapter 1 of this Section.

b) Method

The same method was used as in Chapter 1 in this Section, except that a dose of 60 mg of sodium salicylate per kg body weight was used in this study in a dose size of 1.5 cm^3/kg body weight. An 8 by 8 latin square pattern of experimental design was employed and finally no 24 hour blood samples were taken in this study. The experimental design is shown in Table 2.1 and the 8 formulations are represented by the letters A-H. Formulations B, D and E were prepared according to the method described by Lin and Pramoda (1978) and the remainder according to the method described by Stephens and Su (1975) (see Section 2).

All studies were initiated at the same time of day in order to eliminate the effect of circadian variation.

2.3 Results

The mean concentrations of salicylate in the blood samples that were taken from the 8 rabbits at various times after oral administration of the sodium salicylate suspensions are given in Table 2.2. Plots of the mean concentrations versus time are shown in Fig.2.1.

The mean values of $(AUC)_o^9$, PC and PT are given in Table 2.3, which also shows the apparent viscosity of each formulation at a shear rate of 100 s^{-1}. The AUCs were calculated by using the trapezoidal rule as described in the previous chapter.

Since rabbit number 7 died in the last time period (i.e. time period 8), the missing values for the three bioavailability parameters (PT, PC and AUC_o^9) were calculated according to the equation 2.1 (Davis, 1954).

Table 2.1 Experimental Design

Rabbit No.	Time Period							
	1	2	3	4	5	6	7	8
1	A	B	C	D	E	F	G	H
2	B	D	H	F	C	A	E	G
3	C	H	E	B	G	D	A	F
4	D	F	B	H	A	G	C	E
5	E	C	G	A	H	B	F	D
6	F	A	D	G	B	E	H	C
7	G	E	A	C	F	H	D	B
8	H	G	F	E	D	C	B	A

The suspensions contained sodium salicylate 4% w/v in

A. fractionated coconut oil (FCO).

B. 20% w/v sucrose in FCO

C. 1% w/v aluminium stearate (50:50 mixture of mono- and distearates) in FCO

D. 20% w/v sucrose + 0.3% w/v Cab-o-sil in FCO

E. 20% w/v sucrose + 1.0% w/v Cab-o-sil in FCO

F. 0.5% w/v aluminium stearate + 0.7% w/v lecithin + 0.35% w/v hydrogenated castor oil + 20% w/v sucrose in FCO

G. 0.5% w/v aluminium stearate + 0.35% w/v hydrogenated castor oil + 20% w/v sucrose in FCO

H. 4.0% w/v aluminium stearate in FCO.

Table 2.2 Mean blood salicylate concentration (mg/100 cm^3) at various times after administration of each oily suspension of sodium salicylate. Each value is the average of 8 experiments.

Formul^n (a) \ Time(hr)	½	1	2	3	4	5	6	9
A	7.9	9.8	11.7	11.3	11.5	10.7	10.1	8.6
B	10.2	12.1	14.0	14.1	13.2	12.7	11.6	9.9
C	7.6	9.9	11.4	12.4	11.9	11.7	11.5	10.3
D	6.9	9.1	12.0	12.7	13.2	12.7	12.5	11.3
E	5.2	7.3	9.5	10.9	11.1	11.0	10.6	9.5
F	6.3	8.9	12.2	12.3	12.5	12.5	12.0	10.4
G	5.7	7.9	10.0	11.5	11.3	11.4	11.6	10.5
H	6.5	8.5	9.9	10.6	10.4	10.4	10.3	9.2

Key: (a) The formulations are as specified in Table 2.1

Fig. 2.1 Mean blood concentration of salicylate versus time following oral administration of different formulations of sodium salicylate as a single dose of 60 mg/kg body weight in 8 rabbits.

●——● = A, ○ = B, ✸ = C, ●-·-·-● = D, ☼ = E, ✲ = F, ✲ = G, and ✲ = H.

Key : see Table 2.1

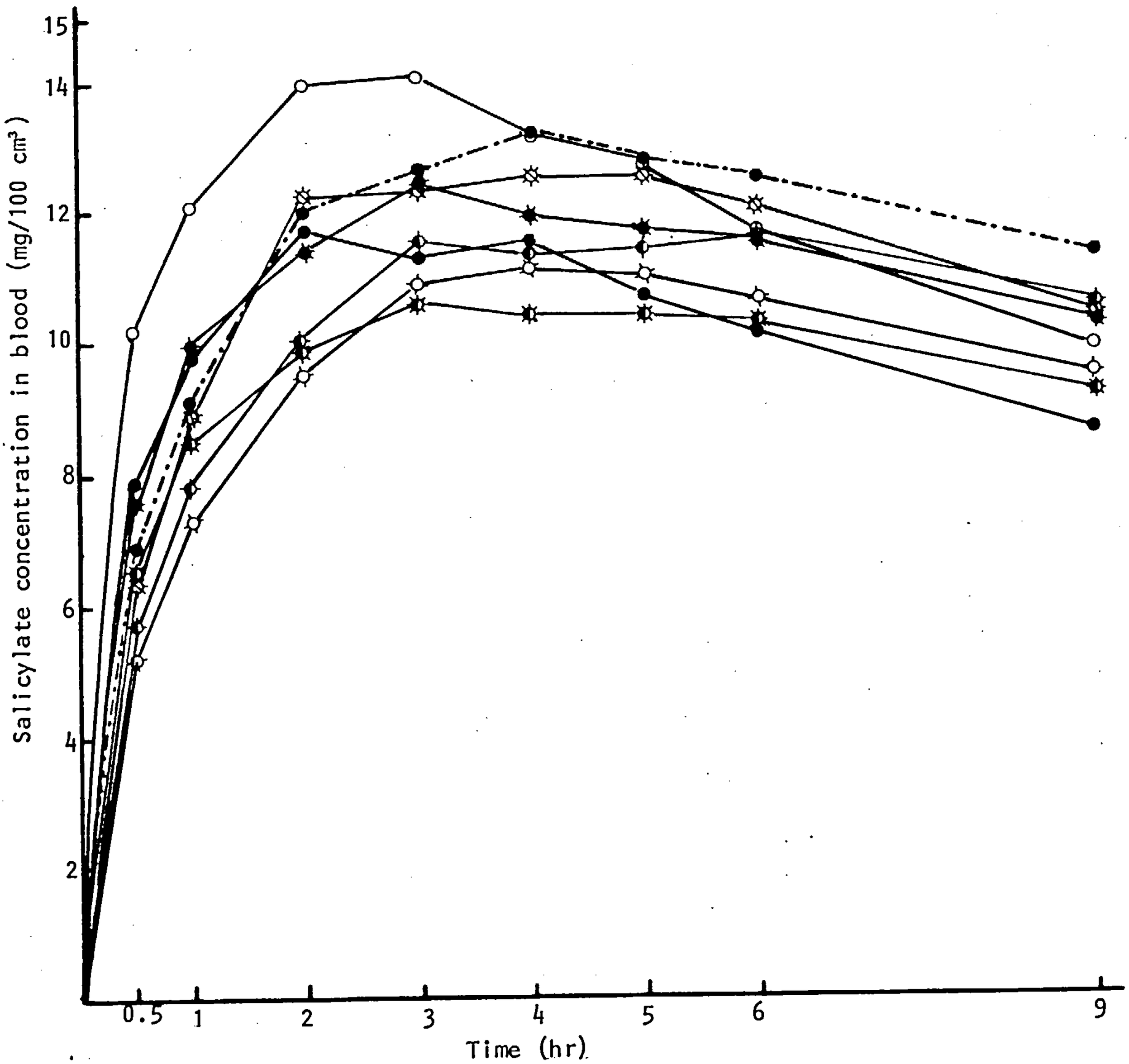


Table 2.3 Mean peak blood concentrations (PC), peak times (PT) and area under blood level versus time curve $(AUC)_o^9$ following oral administration of different formulations of sodium salicylate as a single dose of 60 mg/kg body weight.

Formul[n] (a) / Parameter	A	B	C	D	E	F	G	H
PT hr	2.8	2.8	3.3	4.6	4.3	4.0	5.5	4.5
PC mg/100 cm^3	12.8	14.7	12.8	13.9	12.0	13.8	12.7	11.4
AUC_o^9 mg hr/100 cm^3	89.8	106.0	97.0	102.8	86.1	99.0	91.9	85.4
η_{app} mN s m^{-2} (b)	17.5	51	50	83	131	120	105	144

Key:

a. The formulations are as specified in Table 2.1.

b. η_{app}, apparent viscosity, from Table 1.2, Section 2.

$$(m-1)\ (m-2)\ X = m\ (R+C+T) - 2S \qquad \text{Eq. 2.1}$$

where R = total of known values in row containing X

C = total of known values in column containing X

T = total of known values in treatment values containing X

S = total of all available values

m = number of treatments, columns or rows

X = missing value

The calculation of the missing value for PT is given as an example

R = 24

C = 39

T = 18

and S =249

$$(8-1)\ (8-2)\ X = 8\ (25+39+18) - 2 \times 249$$

$$42\ X = 158$$

$$X = 3.8 \text{ hr} \sim 4 \text{ hr}$$

using the same procedure the calculated values of the PC and AUC_o^9 were 15.7 mg/100 cm^3 and 117.9 mg hr/100 cm^3, respectively.

Analysis of variance (ANOVA) of the results was carried out according to Schefler (1979) to determine the significance of the differences between the mean values of the three bioavailability parameters, PT, PC and AUC_o^9 . The analysis of variance of the PT is given below as an example.

Table 2.4 Individual values of peak time (PT) of salicylate in the blood following oral administration of the 8 formulations according to the Latin square design in Table 2.1.

formulation / rabbit No.	A	B	C	D	E	F	G	H	Total Row
1	1	2	3	4	3	4	4	6	27
2	2	3	1	2	5	2	9	3	27
3	4	3	3	4	4	6	3	6	33
4	4	3	3	9	5	6	3	5	38
5	2	1	6	6	6	2	9	3	35
6	4	3	4	4	4	2	3	2	26
7	2	4	3	4	2	4	4	6	29
8	3	3	3	4	5	6	9	5	38
Total Column	22	22	26	37	34	32	44	36	253

$$\text{Correction factor (C.F.)} = \frac{(\sum X)^2}{n} = \frac{(253)^2}{64} = 1000.141$$

$$\text{Sum of squares total, } SS_{Total} = \sum X^2 - \text{C.F.}$$

$$= (1)^2+(2)^2+(3)^2+.....+(9)^2+(5)^2-1000.141$$

$$= 1225 - 1000.141 = 224.859$$

$$SS_{Row} = \frac{(27)^2}{8}+\frac{(27)^2}{8}+\frac{(33)^2}{8}+\frac{(38)^2}{8}+\frac{(35)^2}{8}+\frac{(26)^2}{8}+\frac{(29)^2}{8}+\frac{(38)^2}{8} - 1000.141$$

$$= 1022.125 - 1000.141 = 21.984$$

$$SS_{Column} = \frac{(22)^2}{8}+\frac{(22)^2}{8}+\frac{(26)^2}{8}+\frac{(37)^2}{8}+\frac{(34)^2}{8}+\frac{(32)^2}{8}+\frac{(44)^2}{8}+\frac{(36)^2}{8} - 1000.141$$

$$= 1053.125 - 1000.141 = 52.984$$

$$SS_{Error} = SS_{Total} - (SS_{Row} + SS_{Column})$$

$$= 224.859 - (21.984 + 52.984) = 149.891$$

Table 2.5 ANOVA[(a)] of PT of salicylate in blood following administration of oily formulationsin 8 by 8 Latin square design.

Source of variation	df[(a)]	SS[(a)]	MS[(a)]	F[(a)]
Rows (rabbits)	7	21.984	3.141	1.06
Columns (treatments)	7	52.984	7.569	2.424
Error	48	149.891	3.123	
Total	62	224.859	-	

The critical value of $F_{7,48}$ at 5% probability level is 2.21. Thus,there is a significant difference between the mean values of the peak times because the critical value is less than the $F_{7,48}$ of the treatment, i.e. 2.424, given in Table 2.5.

(a) The meanings of the symbols are given in Table 1.5 in the previous Chapter.

In order to ascertain the statistical significance of the differences between individual mean peak times it was necessary to carry out further analysis of the results. Therefore Duncan's multiple range test (1955) was applied as follows:

Standard error of a varietal means, $S_m = \sqrt{\frac{MS\ error}{n}} = \sqrt{\frac{3.123}{8}} = 0.625$

The sample size	(2)	(3)	(4)	(5)	(6)	(7)	(8)
The significant studentized ranges, P, for a 5% level test for n_2 =48 degrees of freedom obtained from Duncan's Table :	2.86	3.01	3.10	3.17	3.22	3.27	3.3
The shortest significant range, p x S_m = Rp :	1.788	1.881	1.938	1.981	2.013	2.044	2.063

Means of peak time in rank order:	A	B	C	F	E	H	D	G
	2.8	2.8	3.3	4.0	4.3	4.5	4.6	5.5

The differences between the individual means are set out in the following order: the largest minus the smallest, the largest minus the second smallest, up to the largest minus the second largest; then the second largest minus the smallest, the second largest minus the second smallest, and so on, finishing with the second smallest minus the smallest. Each difference is significant if it exceeds the corresponding shortest significant range; otherwise it is not significant. For example, because G-A is the range of eight means, it must exceed $R_8 = 2.063$, the shortest significant range of eight means, to be significant; because G-B is the range of seven means, it must exceed $R_7 = 2.044$, the shortest significant of seven means, to be significant; and so on. An example is given below to illustrate the picture:

G-A = 5.5 - 2.8 = 2.7 > 2.063 therefore significant at 5% level

G-B = 5.5 - 2.8 = 2.7 > 2.044 therefore significant at 5% level

G-C = 5.5 - 3.3 = 2.2 > 2.013 therefore significant at 5% level

G-f = 5.5 - 4.0 = 1.5 < 1.981 therefore not significant at 5% level

G-E = 5.5 - 4.3 = 1.2 < 1.938 therefore not significant at 5% level

G-H = 5.5 - 4.5 = 1.0 < 1.881 therefore not significant at 5% level

G-D = 5.5 - 4.6 = 0.9 < 1.788 therefore not significant at 5% level

The results can be summarised as:

PT (hr) A B C F E H D G

probability < 0.05

where any two means not underlined by the same line are significantly different, and any two means underlined by the same line are not significantly different.

Using the same methods of analysis for PC and AUC_o^9 values, the following results were obtained:

PC (mg/100 cm³): H E G A C F D B

probability < 0.05

AUC_o^9 (mg/hr/100 cm³) H E A G C F D B

probability < 0.05

2.4 Discussion

The AUC values given in Table 2.3 reflect the relative amounts of salicylate absorbed within 9 hr. A comparison of the AUCs obtained with formulations A and B indicates that the presence of sucrose in the oily vehicle leads to a significant increase in the amount of salicylate absorbed, $p < 0.05$. It is suggested that this increase is caused by the osmotic effect of the sucrose after it is released from the oily phase and dissolves in the aqueous fluids of the GI tract. This osmotic effect could involve two mechanisms, i.e. it could produce an extra delay in the GER (see Chapter 2, Section 1) or it could reduce the loss of water from the GI tract into the tissues (Parsons et al, 1958; Mayersohn and Gibaldi, 1971) thereby producing a greater volume of water into which salicylate can partition.

With regard to the first mechanism osmotic pressure has been shown to have a significant effect on the bioavailability of aminopyrine and dipyrone in the rabbit, phenobarbitone and strychnine in the rat (Kato et al, 1969) and sulphafurazole in the rat (Marvola et al, 1979b). Malone et al (1960) attributed the delay in phenobarbitone absorption, when administered with sucrose solution, to the viscosity of the solution. However, Kato et al (1969) reported that 1% carboxymethylcellulose, which has a viscosity similar to 50% sucrose but a much lower osmotic pressure,

has the same GER as water and 50% sucrose caused a delay in the GER. It was concluded that this delay in GER was due to the osmotic effect of sucrose.

For a highly lipophilic drug, e.g. salicylic acid, the drug availability is dependent on the concentration of drug in solution in the GI fluids, which, in the present system, is in turn dependent on the rate of supply from the oily phase. Grisafe and Hayton (1978) have indicated that the direct absorption of griseofulvin from micellar and oil phases is negligible when compared with absorption from the aqueous phase of an emulsion formulation. Kakemi et al (1972 a and b) also suggested that drugs, (e.g. salicylamide), dissolved in the oil phase of an emulsion and oily solution are absorbed mainly via the aqueous phase and that the transference to this phase from the oil may be the rate limiting step. These workers considered a variety of drugs with different oil : water partition coefficients (i.e. <1, 1 and >1) and concluded that the amount of drug in the aqueous phase, rather than its concentration, is a critical factor in determining the absorption of drugs from emulsions. Since this amount will depend on the relative volumes of the oil and water phases the absolute volume of the aqueous fluids is also a critical factor. The importance of the total volume of water was stressed by Kakemi et al (1972a) particularly in relation to drugs with oil : water partition coefficients of more than unity. Since the apparent partition coefficient of sodium salicylate between oil and 0.1 mole/dm^3 HCl is 38.6 (see Chapter 2, Section 4) it seems that the second possible effect of osmotic pressure, i.e. on the loss of water from the GI tract by membrane uptake, may be important with regard to the bioavailability of salicylate from oily systems. Further evidence that the importance of this second effect is related to the partition coefficient of the drug in question is provided by the results

obtained with ampicillin suspensions (see Chapter 4 in this Section).

It is suggested, therefore, that the enhancement of salicylate absorption produced by the inclusion of sucrose in the oily suspensions of sodium salicylate is mainly due to the effects of osmotic pressure on the uptake of water by the GI membranes and not to any additional delay in GER over that caused by the oil itself.

The in vivo effect of sucrose on the absorption of salicylate from the oily suspensions is paralleled by its effect on the in vitro release of salicylate from the same formulations when using a dialysis method (see Chapter 1, Section 4). The presence of sucrose caused the influx of water into the oily product inside the dialysis sac after an initial lag period. The rate of release of salicylate from the dialysis sac was observed to increase after this lag period.

A comparison of the AUC values for formulations A, C and H suggests that the inclusion of 1% aluminium stearate in the FCO increases the amount of salicylate absorbed but 4% aluminium stearate decreases the amount. However, these differences were not statistically significant. The AUCs for B and H differ significantly thus indicating that sucrose enhances salicylate absorption when compared with aluminium stearate. It should be noted that the apparent viscosities of both formulations are greater than that of the simple oily suspension A, but the apparent viscosity of H is the greatest. The effect of sucrose cannot, therefore, be ascribed to the viscosity of the product unless there is an optimum viscosity beyond which the amount of salicylate decreases as the viscosity is further increased.

A consideration of the AUC values for A,B,D and E suggests that the presence of 1% Cab-o-sil nullifies the effect of the sucrose whereas 0.3% Cab-o-sil allows the effect to be retained. Two plausible mechanisms might be suggested to explain this effect of Cab-o-sil. The

first of these is based on the relatively high apparent viscosity of formulation E which may retard partitioning of the drug between the oily and aqueous phases. The second mechanism arises from the fact that the very small particles of Cab-o-sil produce a large interfacial area and the capability of these particles to form hydrogen bonds, via the silanol groups on their surface, with other compounds (Marshall and Rochester, 1975), e.g. methyl salicylate (Sherriff and Enever, 1979), may lead to a marked adsorption of the drug and so hinder its release and subsequent absorption. Incorporation of 5% colloidal silica (Aerosil) resulted in no release at all of sodium salicylate from fatty suppository bases (Schoonen et al, 1976). In fact, the second mechanism is suggested to be the most likely explanation, since the in vitro adsorption studies verified this phenomena (see Chapter 2, Section 4). Since there is a finite number of silanol groups in the gels, a point will be reached where they will be all involved in particle-particle or drug-particle interactions when the concentration of the colloidal silica is too small (Sherriff and Enever, 1979). This may explain why 0.3% Cab-o-sil was not able to retard the bioavailability of sodium salicylate in formulation (D).

Finally, it should be noted that the amount of drug absorbed from formulation F, which corresponds to the vehicle described in the patent assigned to Eli Lilly and Co. (Stephens and Su, 1975), is intermediate between formulations A and B and is not significantly different from either of them.

The results of Duncan's test show that none of the PC values obtained with formulations containing additives (i.e. B-H) were significantly different at $p > 0.05$ from the simple suspension in oil (i.e. formulation A). The significant differences that are observed

are similar to some of those obtained in the case of the AUC values. Thus:

(i) a comparison of B and E suggests that the increased amount of absorption caused by the presence of sucrose is nullified by the inclusion of 1% Cab-o-sil.

(ii) 20% sucrose with or without 0.3% Cab-o-sil (formulations B and D, respectively) leads to a significant increase in PC when compared with the effect of 4% aluminium stearate (H). In addition, formulation F produced a higher PC than H.

In several cases the formulations produced blood salicylate concentration v. time curves with broad peaks. It was consequently difficult to determine individual values for the peak time (PT). This was particularly so in those curves where the maximum concentrations were obtained at the six hour and nine hour sampling times. In spite of these difficulties no significant differences at $p > 0.05$ were detected between any of the mean PT values for the different formulations with the exception of formulation G, which contained all the ingredients of the Eli Lilly Patent except lecithin (see Table 2.1). However, if the PT and η_{app} values in Table 2.3 are compared it would seem that the PT appears to increase from approximately three hours to four or more hours when the apparent viscosity exceeds approximately 80 mN s m^{-2}. The effect is only significant with formulation G as pointed out above.

It is expected that an increase in viscosity would cause a decrease in the rate of release of sodium salicylate from the oily phase. Ashley and Levy (1973) showed that the absorption of phenolsulphonphthalein was reduced during the first hour after administration when viscous sodium alginate solution was used as the vehicle and Buckwalter and

Dickison(1948 and 1958) reported that peanut oil or sesame oil gelled with aluminium stearate delays the absorption of included drugs when compared with either the oil alone or oil plus beeswax.

A comparison of the AUC, PC and PT values of formulations F and G in which lecithin is respectively present or absent, shows that although the differences were not significant (at $p > 0.05$), lecithin appears to increase the amount and rate of absorption of sodium salicylate. The apparent viscosity of the lecithin containing product is the higher. However, it may be suggested that the surface activity of lecithin promotes absorption either by affecting the permeability of the mucosal membrane or by aiding the emulsification of the oily vehicle in the aqueous GI fluids so that the increase in area of contact between oil and water allows more efficient transfer of drug to the aqueous phase.

The most important conclusion that can be drawn from this study is that the inclusion of 20% sucrose alone in the oily vehicle improves the absorption of sodium salicylate in the rabbit. However, this formulation does not provide very satisfactory physical stability of the suspension. Formulation F (Eli Lilly Patent) appears to offer a suitable compromise between absorption and stability characteristics. The present results also suggest more attention should be paid to the osmotic pressure of drug solutions or suspensions, since the osmotic pressure of hypertonic solutions, particularly, might modify the absorption of drugs from their dosage forms.

It should be borne in mind that the comments made in this Chapter concerning the extent of absorption of salicylate from the various oily formulations are based on AUC measurements that range only from 0 to 9 hours. Bioavailability assessments should really be based on AUC_0^{∞} values and shorter time values are only useful if it can be shown

that they are linearly related to AUC_o^{∞} values. Estimation of AUC_o^{∞}s using Eq. 1.4 that is given in the previous chapter is not very satisfactory because the data presented in the present chapter do not allow the value of k_{el} to be determined with a good degree of accuracy. However, if the mean k_{el} value (0.048 hr^{-1}) that is determined from the results obtained for the oily suspension of sodium salicylate in Chapter 1 of this Section is used instead then the derived AUC_o^{∞} show a reasonably linear correlation with the AUC_o^9 values given in Table 2.3 (correlation coefficient = 0.8047, $p < 0.02$).

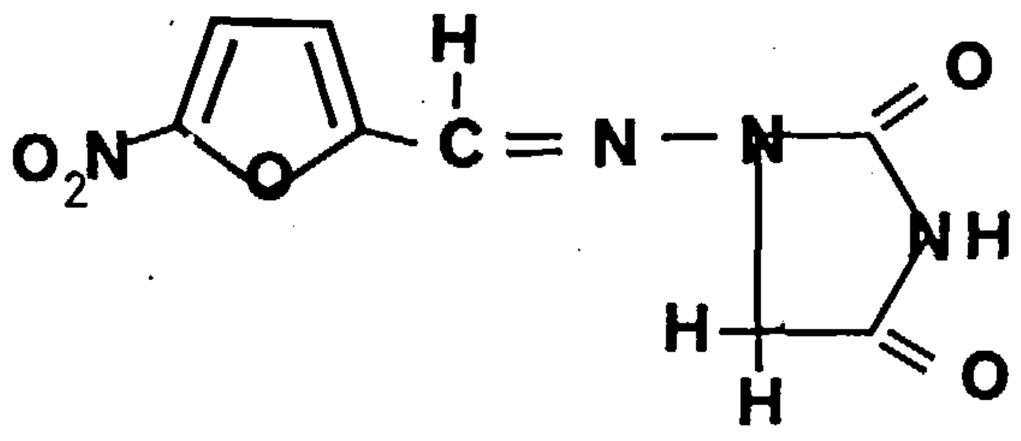
CHAPTER 3

NITROFURANTOIN

3.1 Introduction

(a) Physico-chemical properties

Nitrofurantoin is N-(5-nitro-2-furfurylidene)-1-amino hydantoin or 1- [(5-nitrofurfurylidene)amino] hydantoin having the following chemical structure



Nitrofurantoin is described as lemon-yellow, odourless crystals or fine powder having a bitter taste (Martindale, 1977[a]; Cadwallader and Hung Won Jun, 1976). The drug is a weak acid with a pKa value of 7.2 and a m.p. of 270-272 ^{o}C (The Merck Index, 1976), possessing low aqueous and oil solubility characteristics. Its solubility in water at various temperature and pH conditions has been reported together with its solubilities in different organic solvents by Cadwallader and Hung Won Jun (1976). For example, the solubility in peanut oil is (2.07 mg/100 cm^3) and at 37^{o}C the solubility in water is 17.41 mg/100 cm^3. This latter solubility is pH dependent; e.g. 15.4 mg/100 cm^3 and 37.4 mg/100 cm^3 at pH 1.2 and 7.2, respectively (Bates et al, 1974a).

Nitrofurantoin and its solutions are discoloured by alkali and by exposure to light, and are decomposed upon contact with metals other than stainless steel and aluminium. Since the drug

solutions are photosensitive, all analytical operations must be conducted under subdued light. In addition, well-closed, light-resistant containers should be used for the storage of nitrofurantoin and its preparations. A shelf-life of 5 years is claimed for tablets and suspensions when stored at room temperature in regular glass containers (Cadwallader and Hung Won Jun, 1976).

As might be expected of a compound with limited water solubility, the dissolution of nitrofurantoin is particle size dependent (Stoll et al, 1973), and is also affected by the pH of the dissolution medium (Bates et al, 1974a). Its rate and extent of absorption have been shown to be influenced by the particle size (Paul et al, 1967; Conklin et al, 1969). The dissolution and absorption rates of a 1:5 molar ratio nitrofurantoin-deoxycholic acid co-precipitate were found to be more rapid than those of the drug alone or of its physical mixture with deoxycholic acid (Stoll et al, 1973).

(b) Action and uses

Nitrofurantoin is an antibacterial agent used extensively in the treatment of urinary tract infections (Saunders, 1974c; Cadwallader et al, 1978). Having a broad spectrum of activity, it is effective against all strains of E.coli, S.aureus and enterococci. Antimicrobial concentrations are not reached in the blood but the drug is concentrated in the urine and bactericidal concentrations are achieved. Therefore, urine levels and recoveries, rather than blood levels, are important indicators of bioavailability (Conklin, 1972; Cadwallader et al, 1978). It is most active in acidic urine and if the pH exceeds 8 most of the antibacterial activity is lost. It is given orally in a dose of 50-150 mg 4 times a day (Martindale,

1977a).

Nitrofurantoin occasionally causes nausea, vomiting, drowsiness and headache (Martindale, 1977a). The crystal size of the drug has been found to affect the degree of emesis and the rates of GI absorption and urinary excretion following oral administration (Paul et al, 1967). Nitrofurantoin should be used carefully in patients with marked renal failure.

(c) Absorption, distribution and elimination

Nitrofurantoin is readily absorbed after oral administration (Conklin et al, 1969). The small intestine is the primary and chief site of absorption and high plasma levels are provided within minutes (Buzard et al, 1961; Conklin, 1972). Some absorption also occurs in the colon but none can be demonstrated from the stomach (Buzard et al, 1961). Absorption of nitrofurantoin from the small intestine is rapid and appears to follow the pH-partition theory with little indication of gastric absorption. Only limited drug absorption occurs when nitrofurantoin is administered rectally (Conklin, 1972).

The bioavailability of nitrofurantoin has received a considerable attention in recent years. The general characteristics and experimental criteria for bioavailability testing of nitrofurantoin were discussed in the monograph presented by Cadwallader et al (1978). One of the criteria is that urinary excretion data should be used for assessment of the bioavailability because of the relatively low nitrofurantoin blood levels that result from the very high and rapid excretion of the drug in the urine. In addition, the urinary tract is the actual site of the therapeutic activity. In fact, a linear relationship between the blood levels of nitrofurantoin and its rate of urinary excretion was demonstrated in man (McGilveray et al, 1973). Hence it is possible to determine the rate and extent of absorption of this drug by measuring the rate of appearance and cumulative

amount of unchanged drug in the urine expressed as a percentage of the dose administered. Results obtained from numerous studies, as indicated by Conklin (1972),show that similarities or differences between nitrofurantoin formulations are readily discernible using this approach. Several studies have indicated bioavailability problems associated with the use of commercial nitrofurantoin tablets (McGilveray et al, 1971 and 1973; Meyer et al, 1974). In fact, some products, that met the official compendial requirements, were less bioavailable than the other products studied (Meyer et al, 1974). Particle size of the drug affects the absorption and excretion rates. For example, Paul et al (1967) found that larger crystals of the drug caused less emesis in dogs and slower absorption and excretion in man and rats. Absorption of the drug from tablet formulations is increased considerably in non-fasting as compared to fasting subjects.

Nitrofurantoin is readily and immediately distributed into the extracellular and intracellular compartments and has a half-life in blood of about 20 min. (Buzard et al, 1961; Conklin, 1972; Cadwallader and Hung Won Jun, 1976). The disappearance from the blood apparently follows first-order kinetics. Also there is little evidence for any prolonged binding of nitrofurantoin to either plasma proteins or tissues (Conklin, 1972). The one compartment open model appears to be adequate for describing the kinetics involved in nitrofurantoin absorption and elimination (Conklin, 1972; McGilveray et al, 1971).

Between 30-50% of an orally or intravenously administered dose of nitrofurantoin can be recovered intact from the urine of man and animals (Cadwallader et al, 1978). These values suggest that nitrofurantoin undergoes metabolic transformation in the body to a

significant extent. The possible metabolic pathways of nitrofurantoin are not completely elucidated in the literature. However, it would follow somewhat similar pathways in metabolism to that for nitrofurazone, which undergoes reduction of the nitro group and hydrolysis of the azomethane linkage. It is degraded by all tissues of the body (except blood) into inactive metabolite, which may colour the urine brown (Cadwallader et al, 1978). Nitrofurantoin is excreted in the kidney by glomerular filtration and is both secreted and reabsorbed in the tubules. Nitrofurantoin clearance in the kidney is handled by a weak-acid transport system, which is influenced by pH. Urinary recovery of nitrofurantoin is linearly related to creatinine clearance, and little or no drug is excreted by patients with marked renal failure. Urinary excretion and biotransformation appear to be mainly and equally responsible for the elimination of nitrofurantoin (Buzard et al, 1961; Conlin, 1972; Cadwallader et al, 1978). Nitrofurantoin is also excreted to a lesser extent in the bile (Conklin and Wagner, 1971).

3.2 Experimental

(a) Materials

Details of the sources of the materials and the methods of preparation of the vehicles are given in Section 2. Nitrofurantoin powder was obtained from Sigma Chemical Co., England.

(b) Methods

Nitrofurantoin powder was sieved and the 240/300 portion (53-63 μm) was used to prepare 0.1% w/v suspensions in the following 8 vehicles using the same methods and experimental design as those described for sodium salicylate in Chapters 1 and 2 in this Section,

respectively. The 8 formulations (vehicles) are presented by the letters A-H.

A = Fractionated Coconut Oil (FCO)

B = 20% w/v sucrose in FCO

C = 0.25% w/v xanthan gum in distilled water

D = 0.25% w/v xanthan gum + 20% w/v sucrose in distilled water

E = 1% w/v Cab-o-sil in FCO

F = 0.5% w/v aluminium stearate (50:50 mixture of mono and di-stearate + 0.7% w/v lecithin + 0.35% w/v hydrogenated castor oil + 20% w/v sucrose in FCO

G = 20% w/v sucrose + 0.3% w/v Cab-o-sil in FCO

H = 20% w/v sucrose + 1% w/v Cab-o-sil in FCO

Xanthan gum was used in the aqueous vehicles (C and D) to prevent flocculation of nitrofurantoin. A low concentration (0.25%) was used in order to avoid a high viscosity relative to that of the oily suspension (A). These suspensions were placed in flasks, which were covered by aluminium foil to provide protection from light and left overnight at room temperature. On the following morning they were shaken before removal of the required dose volume into a syringe.

A dose of 10 mg/kg body weight (equivalent to a dose volume of 1 cm^3/100g body weight) was administered to adult male Wistar rats (body weight range 380-569 g) via a catheter inserted into the stomach. The choice of this dose was based on the work of Conklin and Hollifield (1965). The catheter and syringe were flushed out with 1/3 of the dose volume of oil or water, according to the formulation used, before removal from the animal. The rats were starved for 20 hr with free access to water before dosing and were kept in metabolic cages. At the end of this period the collected urine was

used as zero time sample. During the first 10 hr after dosing, the animals had free access to water followed by a liquid diet containing 5% w/v glucose and 0.05% w/v sodium chloride for up to about 36 hr. This was the maximum period over which nitrofurantoin was detectable in the urine obtained from any of the rats.

The design of this experiment was based on an 8 x 8 latin square identical with that employed for the sodium salicylate suspensions in the previous chapter. A minium "wash-out" period of 7 days was allowed between successive experiments.

Due to the difficulties of obtaining urine samples, it was decided to collect them over the following periods: 0-4, 4-8, 8-24, 24-27, 27-30, 30-32, 32-34, 34-36 hrs after dosing. Samples were collected during the later periods only if the previous sample had been shown to contain any nitrofurantoin. All samples were assayed immediately after collection by the method of Conklin and Hollifield (1965) and all studies were initiated at the same time of day in order to eliminate the possible effect of circadian variation.

The assay method of Conklin and Hollifield(1965) was as follows: To 1 cm^3 of urine and 4 cm^3 of HCl (0.1 mole/dm^3) in a test tube 10 cm^3 of nitromethane (B.D.H.) were added. The contents of the tube were mixed vigorously for 2 min. and centrifuged. 4 cm^3 of the nitromethane (bottom layer) were removed and transferred to another test tube. At this point some of the samples may be cloudy in appearance. If so, the tube containing the solvent was placed under warm tap water for about 1 min. To the nitromethane extract 0.5 cm^3 of 0.04 mole/dm^3 Hyamine in absolute methanol (Packard Instrument Co., Inc.) was added and the contents were mixed and allowed to stand for at least 1 min. The concentration of the nitrofurantoin-Hyamine

complex was determined by direct spectrophotometry at 400 nm using nitromethane, that had been run through the procedure with 1 cm^3 of water and 4 cm^3 of HCl, as a blank. The absorbance of each sample was determined within 30 min. after the addition of the Hyamine solution.

The drug concentrations in the urine samples were determined using a calibration curve prepared by the addition of known amounts of nitrofurantoin to rat urine as follows:

50 mg of nitrofurantoin were dissolved in 50 cm^3 of N, N-dimethyl-formamide (Sigma Chemical Co.) and further dilution of the solution was made with water to obtain different concentrations. 1 cm^3 of each of these standard solutions was mixed with 1 cm^3 of rat urine and 3 cm^3 of HCl (0.1 mole/dm^3) and the rest of the previous procedure was followed. Table 3.1 shows the concentrations and the corresponding absorbance values of the standard solutions.

Table 3.1

Concentration $\mu g/cm^3$	Absorbance (A)	A-Ao
0	0.025	
5	0.065	0.04
10	0.098	0.073
20	0.176	0.151
30	0.25	0.225
60	0.474	0.449
80	0.622	0.597
100	0.769	0.744

The regression coefficient (b) of a plot of (A-Ao) versus concentration was calculated and the concentrations of nitrofurantoin in the urine samples were obtained using Eq. 3.1 as described in Chapter 1 of this Section.

$$X = \frac{Y - 0.0018}{0.00744} \qquad \text{Eq. 3.1}$$

3.3 Results

The volumes of the urine samples collected at various times in the post-administration period were measured (in cm^3) and the concentrations of nitrofurantoin in 1 cm^3 of each sample were determined. From the products of the volumes and concentrations the amounts of drug excreted in each time period were calculated. Successive addition of these amounts yielded the cumulative amount excreted. The amount of drug excreted during each time period together with the cumulative amount excreted were expressed as a percentage of the dose administered. The mean values of these percentages are shown in Table 3.2, which also shows the apparent viscosity of each formulation at a shear rate of 100 s^{-1} and temperature of 37^{o}C. Analysis of variance and Duncan's multiple range test were carried out for the values obtained for the amount of the drug excreted during the first 4 and 8 hr and the cumulative amount excreted (expressed as percentage of the dose), according to the methods given for sodium salicylate in the previous chapter, and the results of these analyses are shown in Table 3.3. The mean values given in Table 3.3 are illustrated diagramatically by Fig. 3.1 - 3.3.

4.4 Discussion

The results given in Fig. 3.1 - 3.3 and Tables 3.2 and 3.3 indicate that nitrofurantoin is excreted in the urine, and therefore absorbed, at both a faster rate and to a greater extent when administered as an aqueous rather than as an oily suspension. This statement is based on the concept that the only way that the drug can get into the urine is via the blood (Dittert and Di Santo, 1978).

With regard to the rate of absorption, the results show that when nitrofurantoin was administered in the two aqueous formulations

Table 3.2 Amounts of nitrofurantoin excreted (as % dose) during different sampling times after administration of nitrofurantoin suspensions (0.1% w/v) as a single dose in an 8 x 8 latin square. Each value is the mean of 8 experiments.

Formulation (a) \ Time (hr)	Nitrofurantoin excreted (% of dose)									
	0-4	4-8	8-24	24-27	27-30	30-32	32-34	34-36	Total	η_{app} mN s m^{-2} (b)
A	7.8	2.9	8.3	0.6	0.3	0.1	0.1	0.05	20.2	17.5
B	9.1	4.0	9.4	0.1	0	-	-	-	22.6	51
C	21.7	8.4	1.6	0 (c)	-	-	-	-	31.7	33
D	22.8	5.4	3.3	0	-	-	-	-	31.5	38
E	7.8	5.7	6.6	0.1	0	0	0	-	20.2	58
F	5.4	2.9	9.7	0.4	0.2	0	0	-	18.6	120
G	8.9	4.3	7.8	0.05	0	0	-	-	21.1	83
H	5.1	5.4	9.4	0.7	0.3	0.05	0	-	21.0	131

Key: (a) the formulations are as specified on page 146.

(b) η_{app}, apparent viscosity, from Table 1.2, Section 2.

(c) recorded zero values indicate that the mean value < 0.05.

Table 3.3. Results of the analysis of variance and Duncan's multiple range test on the values obtained for the amount of the drug excreted during the first 4 hr and 8 hr and the cumulative amount of nitrofurantoin excreted (as % dose) after administration of nitrofurantoin suspensions (0.1% w/v) in various formulations to rats in an 8 x 8 latin square.

0.4 hr	Formulation(a)	H	F	A	E	G	B	C	D
	Mean % dose excreted (b)(c)	5.1	5.4	7.8	7.8	8.9	9.1	21.7	22.8
4-8 hr	Formulation(a)	F	H	A	B	G	E	D	C
	Mean % dose excreted (b)	8.3	10.5	10.7	13.1	13.2	13.5	28.2	30.1
	(c)	F	H	A	B	G	E	D	C
Cumulative amount excreted	Formulation(a)	F	A	E	H	G	B	D	C
	Mean % dose excreted (b)	18.6	20.2	20.2	21.0	21.1	22.6	31.5	31.7
	(c)								

Key: (a) The formulations are as specified on page 146.

(b) Any two means not underscored by the same line are significantly different ($p < 0.01$). Any two means underscored by the same line are not significantly different.

(c) As for (b) except that $p < 0.05$.

Fig. 3.1 Amount of nitrofurantoin excreted (as % dose) during the first 4 hr after oral administration of various suspension formulations. Each value is the average of results obtained in 8 rats.

Key: the formulations are as specified on page 146.

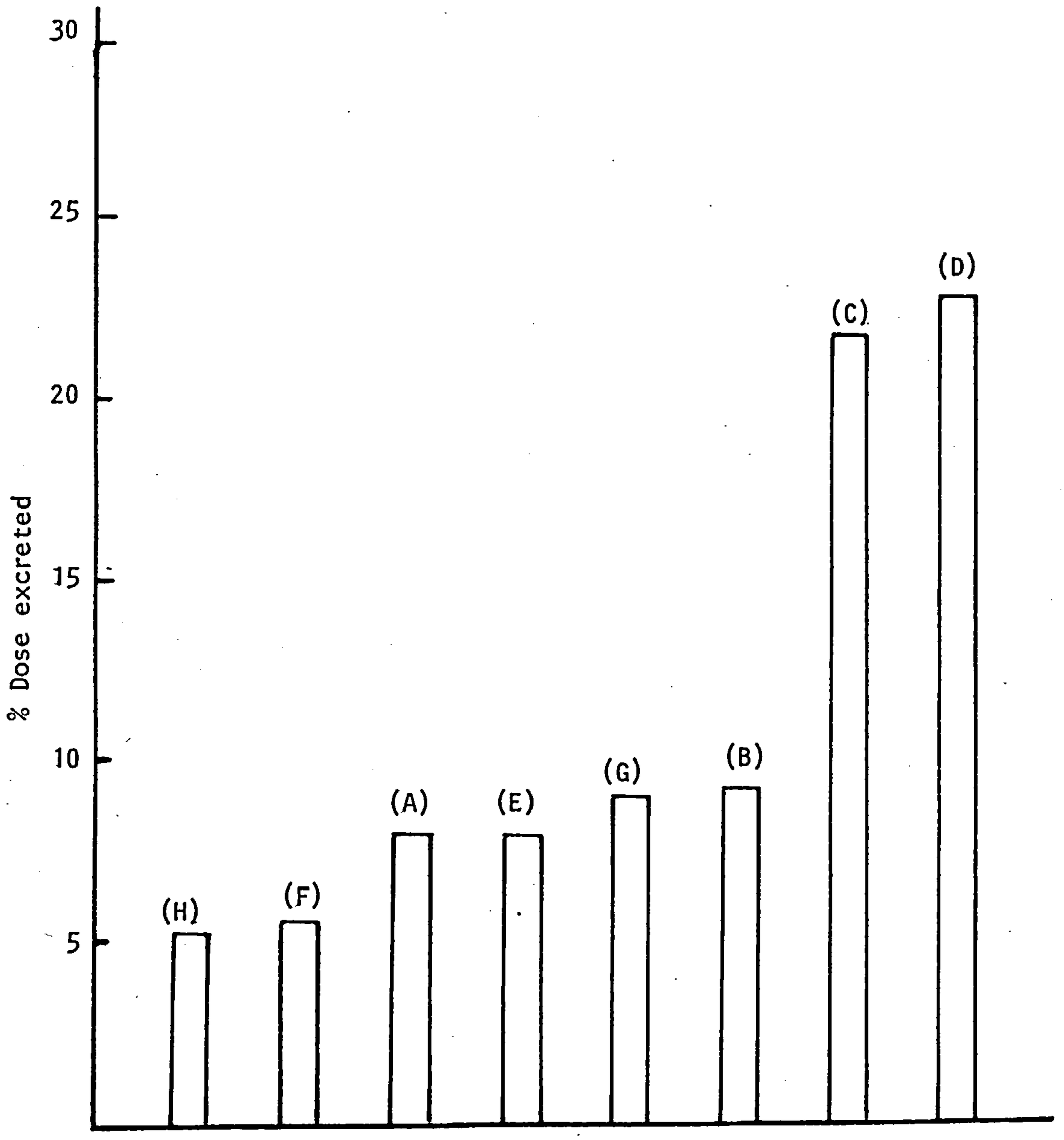


Fig.3.2 Amount of nitrofurantoin excreted (as % dose) during the first 8 hr after oral administration of various suspension formulations. Each value is the average of results obtained in 8 rats.

Key: the formulations are as specified on page 146.

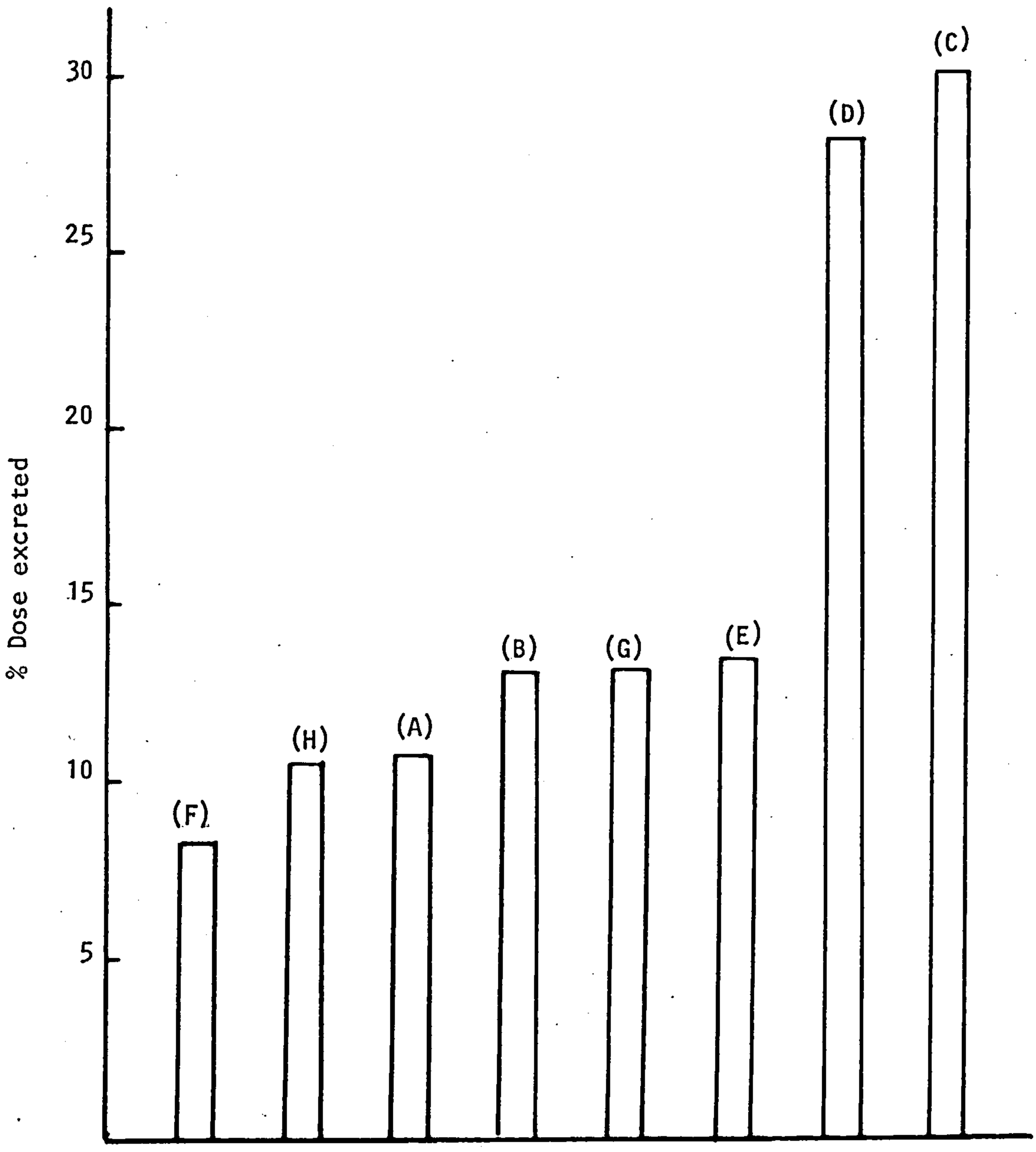
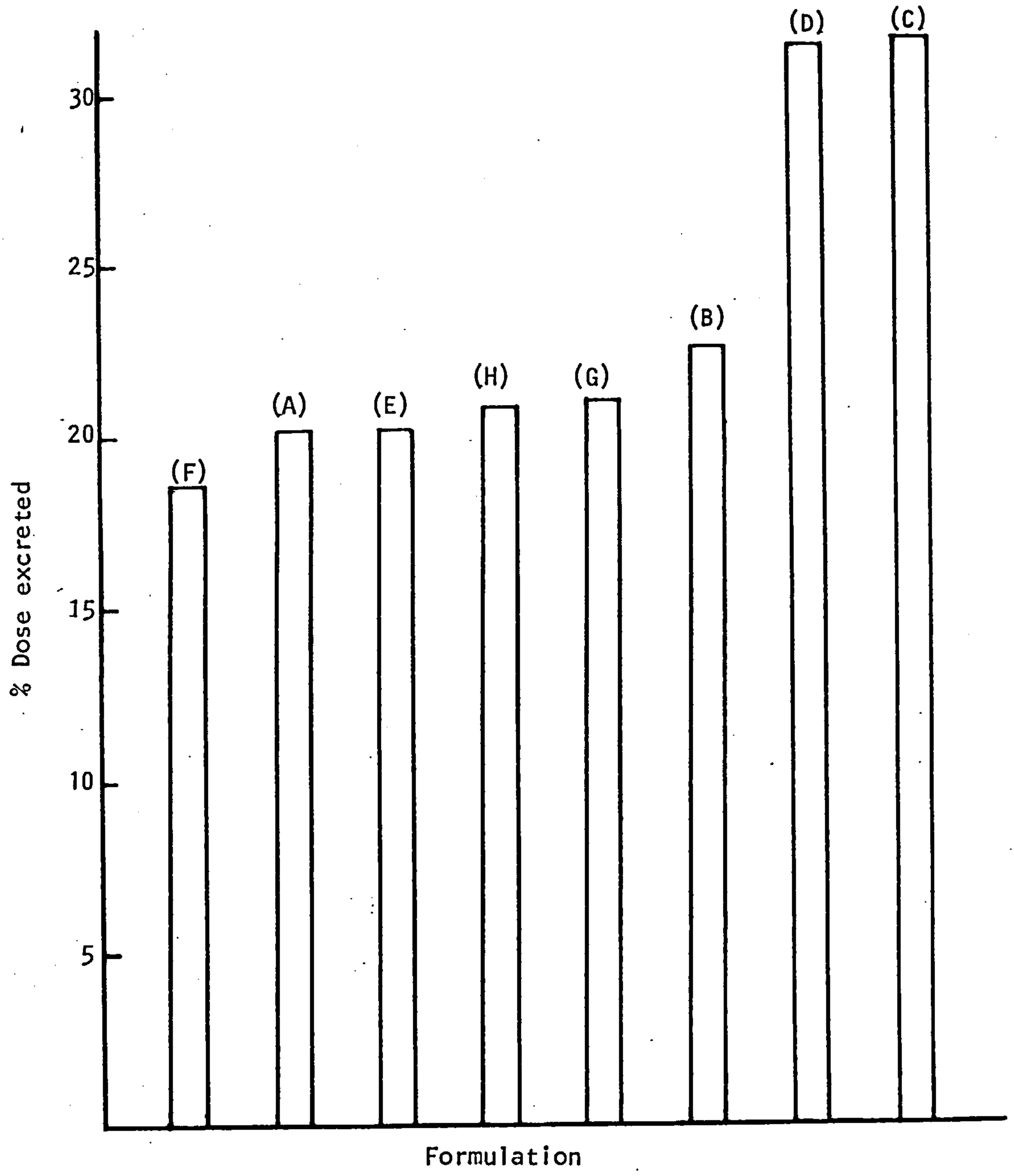


Fig. 3.3 Total amount of nitrofurantoin excreted (as % dose) after oral administration of various suspension formulations. Each value is the average of results obtained in 8 rats.

Key: the formulations are as specified on page 146.



C and D then 70% and 73%, respectively, of the total amount excreted appeared in the urine within 4 hours. After 8 hr the respective values were 95% and 90%. In contrast, the ranges of values obtained using the oily formulations were 24-41% after 4 hr and 45-66% after 8 hr. Statistical analysis indicated that these differences between the aqueous and oily formulations were highly significant ($p<0.01$). The most likely explanation for this difference is that the delay in the GER brought about by the oil (see Chapter 2, Section 1) consequently delays the appearance of the drug in the small intestine, which is regarded as the chief site for absorption of nitrofurantoin (Buzard et al, 1961; Conklin, 1972). These results are in agreement with those which showed that delay in the GER, brought about by using a very high viscous aqueous suspension (Seager, 1968; Soci and Parrott, 1980) or by the presence of food with a commercial tablet (Bates et al, 1974b), decreased the rate of nitrofurantoin absorption. The above explanation is supported by Heading et al (1973), who found that delay in GER slows the absorption of paracetamol, a very weak acidic drug with a pKa value of 9.5, because of the slower appearance of the drug in the small intestine.

The variations in the extent of absorption of nitrofurantoin in the different formulations are reflected by the total amounts of nitrofurantoin that are excreted in the urine (Conklin, 1972; Cadwallader et al, 1978). These amounts, expressed as a percentage of the dose, are given in Table 3.2. Statistical analysis shows that the 1.4 - 1.7 fold differences in the amounts excreted after administration of the aqueous formulations, when compared with those excreted after administration of the oily products, are significant at $p<0.01$.

This finding is opposite to that observed when suspensions of

sodium salicylate were compared with aqueous solutions of that drug, since the extent of absorption of salicylate was greater from the oily formulations (see Chapter 1 in this Section). This difference can be explained on the basis of the pKa values of the two drugs, their sites of absorption from the GI tract and the effect of oil on the rate of gastric emptying. Thus, the ratio of unionized:ionized salicylic acid (pKa = 3),produced when sodium salicylate enters the acidic environment in the stomach, will be considerably greater than the ratio that will exist in the small intestine. The pH-partition theory therefore indicates that the stomach is a favourable site for salicylate absorption (see part 1.3.1, Section 1). Although the areas of absorbing surface in the stomach and intestine have a considerable influence on the relative absorption of drugs from these two sites, in the case of drugs, such as salicylic acid, it is reasonable to suggest that absorption from the stomach provides a significant contribution to the total absorption (see Chapter 1 in this Section). Consequently, an increase in the residence time of salicylate in the stomach is likely to favour an increase in the total amount of drug absorbed from a particular formulation. Oils are known to delay the rate at which the stomach empties (see Chapter 2, Section 1) and oily formulations may, therefore, be expected to enhance the extent of salicylate absorption when compared with aqueous formulations.

In the case of nitrofurantoin (pKa = 7.2) there is a little difference in the degrees of ionization in the stomach and intestine; i.e. 99.99% and 94.06%,* respectively. Thus, bearing the area of the absorbing surfaces in mind, the small intestine is the site for optimum absorption of nitrofurantoin and little contribution to the total amount absorbed is provided by absorption from the stomach. In

* unionized in the stomach (pH1) and duodenum (pH6)

fact, Buzard et al (1961) and Conklin (1972) reported that the small intestine is the chief absorption site of nitrofurantoin and its absorption could not be demonstrated from the stomach. For the same reason, Heading et al (1973) found a significant decrease in the extent of paracetamol absorption when GER was delayed.

Furthermore, nitrofurantoin is degraded by all the tissues of the body (except blood) into inactive metabolite (Cadwallader et al, 1978). This aspect was studied extensively by Buzard et al (1961), who reported that tissues, including the small intestine, are capable of destroying nitrofurantoin at a rate ranging from 1.6 to 7.1 µg/g/min. at body temperature. Cramer (1947), Asnis et al (1952) and Beckett and Robinson (1956) reported that nitrofurantoin entering into the intestinal tract would be subject to enzymatic destruction by intestinal flora. In addition, gastric atrophy permits increased numbers of micro-organisms to pass into the small intestine. Similarly, reduction in intestinal motility results in overgrowth of these flora (see part 1.4.3d, Section 1). Bearing the above considerations in mind, it is suggested that the decrease in the bioavailability of nitrofurantoin is due to the decrease in the gut transit rate brought about by the oil.

Bates et al (1974b) found that food increased the extent of nitrofurantoin absorption from capsules containing macrocrystalline drug. Similar results were obtained by Rosenberg and Bates (1976). They ascribed this effect to the decrease in GER brought about by the viscosity or lipid content of the food, and suggested that such a decrease in GER would allow a greater fraction of the drug to dissolve in the gastric fluids because of an extension of the residence time in the stomach. However, Soci and Parrott (1980) reported that when nitrofurantoin was administered in a very viscous

aqueous suspension the rates of absorption and urinary excretion were slowed but the extent of absorption did not decrease. These results are in conflict with those of Seager (1968) and Barzegar-Jalali and Richards (1980) with regard to the extent of absorption. Seager (1968) found that the amount of drug excreted by humans in 6 hr was significantly reduced ($p < 0.01$) and the biological availability of the drug was impaired by inclusion of 5% w/v methylcellulose in the suspension. Barzegar-Jalali and Richards (1980) observed that the amount of nitrofurantoin excreted in the urine, expressed as percentage of the dose administered to rat, decreased as the viscosity of the suspension medium increased and reported an approximately 2 fold variation between the two extremes of the range of the amount excreted and ascribed this result to the delay in GER, which was caused by the different viscosity enhancing agents.

The results obtained in this study appear to support those of Seager (1968) and Barzegar-Jalali and Richards (1980) and conflict with those of Bates et al (1974b), Rosenberg and Bates (1976) and Soci and Parrott (1980) with regard to the extent of absorption, although they do agree with the results of Bates et al (1974b) and Soci and Parrott (1980) with regard to the rate of absorption. However, Bates et al (1974b) were unable to find any significant enhancement in the extent of absorption from a commercial tablet containing micro-crystalline drug and Rosenberg and Bates (1976) could not find any significant enhancement in the case of commercial nitrofurantoin suspension and a microcrystalline tablet ($p > 0.05$). The only significant enhancement involved the macrocrystalline drug and those dosage forms that gave poor bioavailability in fasting subjects. For example, fasting subjects absorbed similar amounts of nitrofurantoin

from microcrystalline tablet and aqueous suspension, but absorbed significantly less drug from the macrocrystalline drug capsule. The authors (Rosenberg and Bates, 1976) suggested that the microcrystalline tablet was well formulated. However, when nitrofurantoin was assessed in non-fasting subjects, these 3 commercial dosage forms were bioequivalent. It is suggested, therefore, that delay in GER would have an enhancement effect only with those formulations having poor bioavailability in fasting subjects, since it allows a longer time for dissolution of these dosage forms in the GI tract and consequently a better bioavailability. In the case of suspension and well formulated tablet, since dissolution of a significant fraction of the drug occurs fairly rapidly, further delay in GER would have no effect. This suggestion is also supported by Soci and Parrott (1980), who could not find any significant difference in the extent of nitrofurantoin absorption when GER was delayed using a very viscous aqueous suspension compared with a simple aqueous suspension as a reference ($p > 0.05$).

Furthermore, Bates et al (1974b), Rosenberg and Bates (1976) and Soci and Parrott (1980) conducted their experiments in humans, while this study was conducted in the rat. It is possible, therefore, that a difference between the species could offset the similarities in the absorption (Barr, 1972). The rat differs from other species because it does not have a gall bladder (Green, 1963; Williams et al, 1965). It is possible that some absorption of nitrofurantoin could take place in the gall bladder itself (Williams et al, 1965), since nitrofurantoin is excreted to some extent in the bile (Conklin and Wagner, 1971), and this could be a possible explanation of why the extent of absorption of nitrofurantoin, when administered in a

suspension, did not decrease significantly when GER was delayed in humans.

It must be pointed out that enzymatic degradation of nitrofurantoin should be taken into account when a delay in GER occurs for any reason, e.g. food, viscosity enhancing agents or lipids. However, Bates et al (1974b), Rosenberg and Bates (1976) and Soci and Parrott (1980) did not take this point into account in their assessment of the extent of bioavailability.

In addition to the species dependent effect mentioned above it is possible that the enzymatic degradation of nitrofurantoin could also provide the basis of a more detailed explanation of the different effects observed in this study and in previous ones (Seager, 1968; Bates et al, 1974b; Rosenberg and Bates,1976; Soci and Parrott, 1980; Barzegar-Jalali and Richards, 1980). This explanation would depend on the fact that a delay in GER would allow a longer period for the dissolution of undissolved drug and would also provide, not only a longer period for a degradation to occur in, but also a greater amount of drug in solution and consequently available for degradation. These increased extents of dissolution and degradation would have opposing effects with respect to the bioavailability of nitrofurantoin. It therefore seems reasonable to suggest that an optimum bioavailability would be obtained if the degree of dissolution of the administered nitrofurantoin was sufficient to ensure reasonable absorption when it reaches the small intestine but it should not be excessive otherwise an increasing fraction of the dose will be degraded before absorption can occur. If this suggestion is correct it means that there will be a critical rate of dissolution for optimum bioavailability. Thus, if the actual dissolution rate is less than the critical value then a

reduction in GER would be expected to enhance the extent of nitrofurantoin absorption as Bates et al (1974b) observed with capsules containing macrocrystalline drug, which exhibited an 80% increase in the extent of bioavailability when the GER was decreased. Furthermore, the closer the actual dissolution or release rate approaches the critical value the lower the effect of reduced GER would become because the effect of degradation would start to cancel the effect of enhanced dissolution rate. Thus, the bioavailability of a microcrystalline nitrofurantoin tablet was only increased by a statistically insignificant 30% when the GER was decreased by the administration of food (Bates et al, 1974b) and that of a microcrystalline suspension was unchanged (Rosenberg and Bates, 1976).

Extending the suggestion to the present results would indicate that the oily vehicle delays the GI transit time to such an extent that, although release of nitrofurantoin into solution in the aqueous gut fluids will have time to occur, the extent of enzymatic degradation will be appreciable and consequently the degree of absorption of intact drug will be decreased when compared with that obtained using an aqueous vehicle.

There was no significant difference between any of the oily formulations, neither during the first 4 and 8 hr nor between the total amount excreted, except that the amount excreted during the first 8 hr post-administration of formulation (F), which corresponds to the vehicle described by the patent of Stephens and Su (1975), was lower than the amounts obtained using the other oily formulations ($p < 0.05$) (Table 3.3 and Fig. 3.2). A consideration of the viscosity of the formulation (F) (Table 3.2) indicates that it has the second highest viscosity of all the formulations. However, these remaining

formulations show no significant differences in comparison to formulation (A), the simple oily suspension, although their viscosities are higher than (A) and include formulation (H), which has a higher viscosity than F. In addition, no significant difference was observed between the extent of absorption from any of the oily formulations including F ($p>0.05$) (Table 3.3). These results suggest that the viscosity has no additional effect on the extent of absorption of nitrofurantoin from the oily vehicles used in this study and the effect of the oil on GER is predominant.

The inclusion of sucrose in the oil (B) had no significant effect on the rate and extent of nitrofurantoin absorption ($p>0.05$) (Table 3.3) thus suggesting that the delaying effect of osmotic pressure on GER (see Chapter 2, Section 1) had no significant effect in this study. This is supported by comparison of the results obtained with the two aqueous formulations (C and D). The lack of effect of sucrose on nitrofurantoin absorption differs from the situation that was observed with salicylate in the previous chapter where osmotic pressure appeared to cause a significant effect on bioavailability. This latter effect was explained on the basis of the high oil/0.1 mole/dm^3 HCl partition coefficient of salicylate. In the case of nitrofurantoin this partition coefficient is only 0.48 (see Chapter 2, Section 4).

A comparison of formulations A, B, E, G and H (Tables 3.2 and 3.3) suggests that neither 0.3% nor 1.0% Cab-o-sil has any significant effect ($p>0.05$) on the bioavailability of nitrofurantoin in the oily vehicle. The higher concentration, i.e. 1% w/v, nullified the increasing effect of sucrose on the bioavailability of sodium salicylate, when given in the formulation which corresponds to formulation (H) in this study, and the difference was significant ($p<0.05$). It was

suggested (previous chapter) that this effect was possibly due to adsorption of salicylate onto the large surface area of the Cab-o-sil by hydrogen bonding. In vitro studies have confirmed this possibility (see Chapter 2, Section 4). However, the absorption of nitrofurantoin is not altered by the inclusion of the Cab-o-sil. Neither the viscosity (as mentioned above) nor adsorption would seem to have an effect in this study. In fact, in vitro adsorption studies did not detect any appreciable adsorption of nitrofurantoin onto Cab-o-sil (see Chapter 2, Section 4).

In summary, the rate and extent of absorption of nitrofurantoin was decreased significantly by the use of an oily rather than an aqueous vehicle. In addition, enhanced osmotic pressure and increased viscosity did not produce any significant effects on the absorption. It is therefore suggested that the decreased bioavailability is caused by the delaying effect of the oil on the GER and this effect predominates in all the oily formulations used in this study.

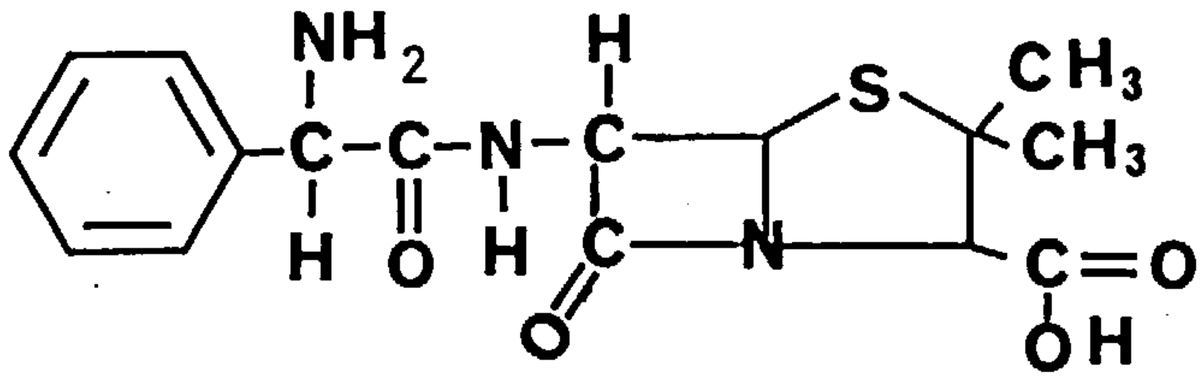
CHAPTER 4

AMPICILLIN

4.1 Introduction

(a) Physico-chemical properties

Ampicillin is D-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo(3.2.0)heptane-2-carboxylic acid. It is also known as (6-(2-amino-2-phenylacetamido)penicillanic acid) and has the following chemical structure.



It is a free-flowing, white crystalline powder with an odour characteristic of penicillin and a bitter taste (Ivashkiv, 1973; Harvey, 1975; Martindale, 1977b).

Ampicillin has 2 pKa values, 2.6 and 7.24 (Ivashkiv, 1973). It has an isoelectric point at pH 4.95 and the water solubility-pH profile shows a U-shaped curve with the minimum solubility at this pH.(Hou and Poole, 1969; Tsuji et al, 1978). Ampicillin is almost insoluble in organic solvents, e.g. alcohol, ether and fixed oils (Marsh and Weiss, 1967; Martindale, 1977b). It gave an ethylacetate-water partition ratio of 0.044, which is not affected by pH (Hou and Poole, 1969). They concluded that ampicillin is more hydrophilic than lipophilic and behaves like an ionic amino acid molecule.

Ampicillin is an acid stable antibiotic (Saunders, 1974b). Hou and Poole (1969) pointed out that ampicillin, by the presence of its side chain amino group, is at least 200 times more acid-stable than penicillin G. Gastric destruction is probably not significant in

terms of ampicillin stability (Jusko et al, 1978).

Ampicillin exists in anhydrous and trihydrate forms (Austin et al, 1965). The solubilities of these forms of ampicillin in 26 different solvents at room temperature (21°C) have been reported by Marsh and Weiss (1967), who quoted values of > 2% w/v for the solubilities in HCl (0.1 mole/dm³) and 1.0% and 0.756% for the solubilities of the anhydrous and trihydrate forms, respectively, in water. Poole et al (1968) obtained 1.0% and 0.8% for these latter two values at 37°C. The significance of the state of hydration of ampicillin will be discussed further in part (c) of this introduction.

(b) Action and uses

Ampicillin has a wide range of antibacterial effects comparable with benzylpenicillin. Given orally in doses of 50-100 mg/kg/day, ampicillin is freely absorbed and maintains a bactericidal level in the plasma for 4 hours or more. It is rapidly excreted, in very high concentration, in the urine (Stewart et al, 1961).

The wide range of the antibacterial effects of ampicillin has been reported due to the presence of the NH_2 group which increases activity against Gram-negative bacilli. It is effective against Staph. aureus at concentrations of 0.05 μg/cm³, it is acid stable and can, therefore,be used orally. However, it is sensitive to penicillinase and so is ineffective against organisms, which have developed resistance to penicillins by increased production of this enzyme (Saunders, 1974b).

Ampicillin is used in the treatment of infections of the respiratory tract and is especially effective where Haemophilus influenzae is the causative organism. It is also employed in the treatment of infections of the urinary tract due to Escherichia coli and Proteus mirabilis. Because ampicillin is excreted in high

concentrations in the bile, it has been used in the treatment of infections of biliary and intestinal tracts caused by E.coli, salmonellae and shigella (Harvey, 1975; Martindale, 1977b).

The usual oral dose is 1 to 6 g daily in divided doses every six hours (Martindale, 1977b).

Ampicillin causes the allergic reactions typical of other pencillins. The drug may cause nausea and vomiting, diarrhoea and stomatitis (Harvey, 1975; Martindale, 1977b).

(c) Absorption, distribution and elimination

Ampicillin is relatively stable in the acid gastric secretion and is well absorbed from the GI tract after oral administration (Acred et al, 1962; Martindale, 1977b; Jusko et al, 1978). The absorption usually occurs within two hours after oral administration of ampicillin capsules and suspensions. Peak plasma concentration has been reported to be at about 1 to 2 hours after oral administration of the drug (Acred et al, 1962; Bear et al, 1970; Hultberg and Backelin, 1972). However, large variations (20-70 percent of the dose) occur in the actual amount absorbed, primarily because of the intrinsic differences in GI absorption of ampicillin among subjects (Jusko et al, 1978).

Ampicillin is eliminated from the body very rapidly with a half-life of about 1.1 to 1.3 hours in adult patients with normal renal function (Dittert et al, 1969; Jusko and Lewis, 1973). Patients with reduced renal function showed higher peak concentrations and later peak times than those with normal functions (Kirby and Kind, 1967; Hultberg and Backelin, 1972). Ampicillin is little metabolised and is excreted almost unchanged in high concentrations in bile and urine (Harrison and Stewart, 1961; Stewart and Harrison, 1961; Acred et al, 1962; Ayliffe and Davies, 1965; Martindale, 1977b; Lund et al, 1976; Sjoqvist et al, 1980).

The bioavailability of ampicillin is relatively easy to assess. The rapid absorption and elimination of the drug limit the duration of blood and urine collection to about 8 hours in normal adults. The relative bioavailability of ampicillin can be assessed using the area under the plasma concentration versus time curve after oral administration of the drug to normal subjects (Jusko et al, 1978).

One of the physicochemical properties that has been thought to affect the bioavailability of ampicillin is its state of hydration (Poole et al, 1968). These latter workers carried out in vivo bioavailability studies on commercially available capsule and suspension dosage forms containing either the anhydrous or trihydrate forms. In spite of the closeness of the aqueous solubilities of the two forms they suggested that the enhanced bioavailability of the products containing the anhydrous material could be ascribed to differences in the dissolution rates of the two forms. This suggestion was supported by their in vitro dissolution rate measurements in water, which were later confirmed by Poole and Bahal (1968). However, Hill et al (1972 and 1975) could not detect any differences in the in vitro dissolution rates in HCl (0.053 mole/dm^3) of the two forms when packed loosely in gelatin capsules. They also showed that the solubility of ampicillin was appreciably greater in dilute acid than in water and that the anhydrous form and the trihydrate were equally soluble in the dilute acid. They suggested that the dissolution rate of ampicillin in HCl is more relevant to its bioavailability from oral products than the dissolution rate in water. They also suggested that the bioavailability differences, that were reported previously by Poole et al (1968), were related to formulation factors and not to the hydration state of ampicillin. This suggestion was confirmed by the in vivo studies of

Hill et al (1975). Other studies have also revealed that commercial capsules containing either form of ampicillin yielded essentially identical bioavailabilities (Mayersohn and Endrenyi, 1973; Loo et al, 1974). In 1973 the FDA suggested the deletion of the term hydrate from the official names of ampicillin dosage forms.

Various reports describing the effect of food on the absorption of ampicillin have been published. While absorption is delayed by the presence of food in the stomach, this does not affect the total amount of ampicillin absorbed (Foltz et al, 1970). The oral absorption of ampicillin has been reported to be good even when taken with food (Bear et al, 1970). However, Ali and Farouk (1980) reported that Sudanese food causes a significant reduction in bioavailability of ampicillin and suggested that the composition of food is critical in this respect.

The degree of binding of the antibiotic to serum macromolecules governs its antibiotic activity; only the unbound drug is effective. In addition, the degree of binding also governs the drug distribution in the body (Saunders, 1974b). This aspect has been the subject of a number of reports (Acred et al, 1962; Rolinson and Sutherland, 1965; Kunin, 1965). Ampicillin shows a lower degree of binding than other penicillins and this binding is greater in human serum than in that of other species studied (Acred et al, 1962; Rolinson and Sutherland, 1965). For example, Acred et al (1962) found that the degrees of ampicillin binding in horse, human and bovine sera were 7.9%, 17% and 17.5%, respectively, whereas the corresponding values for phenoxymethyl penicillin were 39.37%, 51.37% and 68.7%.

The biopharmaceutical aspects of ampicillin in different dosage forms have been extensively reviewed by Ivashkiv (1973), Saunders (1974b) and Jusko et al (1978).

4.2 Experimental

(a) Materials

Ampicillin was obtained from Beecham Pharmaceuticals. Antibiotic medium No. 1 CM 327,adjusted to pH 7.9, and nutrient broth CM1 were obtained from Oxoid Ltd. Bacillus subtilis (No. 8236) was obtained from the N.C.T.C. Details of the sources of other materials and methods of preparation of the suspension vehicles are given in Section 2.

(b) Method

(i) Bioavailability studies

The same method was carried out as in the Chapter 1 of this Section except that a 6 by 6 latin square pattern of experimental design was employed and the sampling times of the blood were 0, ½, 1, 1.5, 2, 3, 4, 6 and 8 hrs after drug administration. The experimental design is shown in Table 4.1 and the 6 formulations are represented by the letters A-F.

The specified amount of the drug was added to the particular vehicle, which had been prepared and left overnight at room temperature, just before dosing the rabbit. Doses of 50 mg/kg body weight were given to rabbits weighing 2.15-3.8 kg in a dose size of 2.5 cm^3/kg body weight.

After collection the blood samples were centrifuged for 5 min and the plasma was stored in a refrigerator until required for the microbiological assay of ampicillin. This assay was commenced on the same day as the bioavailability test, immediately after the last blood sample had been obtained.

(ii) Microbiological assay

A cup-plate assay method was used. This method was very similar to that described by Bennett et al (1966) and the medium, bacterial suspension and other conditions were as described by Arret et al (1971).

Table 4.1. Experimental design

Rabbit No.	Time Period				
	1	2	3	4	5	6
1	A	B	C	D	E	F
2	B	C	F	A	D	E
3	C	F	B	E	A	D
4	D	A	E	B	F	C
5	E	D	A	F	C	B
6	F	E	D	C	B	A

The suspensions used contained ampicillin trihydrate 2% w/v in:

A = Fractionated Coconut Oil (FCO)

B = 30% w/v sucrose in FCO

C = distilled water

D = 30% w/v sucrose in distilled water

E = 30% w/v sucrose + 1.25% w/v Cab-o-sil in FCO

F = 0.5% w/v aluminium stearate (50:50 mixture of mono- and distearate) + 0.7% w/v lecithin + 0.35% hydrogenated castor oil + 30% w/v sucrose in FCO.

A few drops of nutrient broth were added to the freeze-dried sample of B. subtilis and this mixture was used to inoculate a slope of the solid antibiotic medium. After incubation at 37°C for 24 hr the slope was washed with sterile distilled water and the washings were used to inoculate a larger slope. This was incubated at 37°C for 7 days. The sporing organisms were then washed off and the resulting suspension was standardised by adjusting its density to 1/5 of Brown's tube No. 1, so that the number of organisms was in the range 10-100 x 10^6 organisms/cm³. The suspension was then heated to 80°C for 10 min in order to kill vegetative organisms. The spore containing suspension was finally stored at 4°C until required for the assay. Bennett et al (1966) reported that the suspension is stable for 4 weeks when stored under such conditions. Three suspensions were prepared during the course of this study.

When an assay was to be performed molten antibiotic agar medium, whilst at approximately 50°C, was seeded with the standardised B. subtilis suspension (2.5 cm³ of suspension in 250 cm³ of agar). The seeded agar was allowed to set at room temperature for about 1 hr. 36 holes of 10 mm diameter and 10 mm depth were then made in each plate. 20 of these holes were to be filled with 4 replicates of 5 different standard solutions of ampicillin in plasma and the remaining 16 holes were to be filled with the test samples and their replicates. (Single specimens were used for the 0 and 8 hr time samples and duplicate specimens were used for each of the seven intermediate time samples).

Although ampicillin is known to have a low affinity for protein binding (Acred et al, 1962) the standard solutions were prepared with pooled rabbits' plasma in order to eliminate possible variations in antibacterial activity due to this effect (Bennettet al, 1966). A constant volume (0.3 cm³) of pooled plasma was added to 0.2 cm³ amounts

of different concentrations of ampicillin solution in phosphate buffer (0.1 mole/dm³ and pH 7.9 $\pm$ 0.1) to produce 0.5 cm³ quantities of 5 standard solutions, which contained 0.5, 1, 2, 4 and 8 μg/cm³, respectively.

The standard solutions were made on each day of the study after the first or second hourly blood sample had been obtained during the bioavailability test. The standard solutions were then stored along with the unknown plasma samples in a refrigerator so that they were subjected to the same conditions.

The plasma samples and standard solutions were coded and a constant volume of each (50 μℓ) was put into the appropriate hole in an agar plate. Even though the level of each plate was adjusted by means of a spirit-level the distribution of test and standard samples was arranged to compensate for any variation in agar thickness, as well as for the time factor involved in adding the samples to the plate. The plates were then allowed to stand undisturbed for 2 hr at room temperature to allow diffusion of the antibiotic to occur. At the end of this period they were transferred to an incubator and maintained at 37°C for 16 hrs.

The diameter of the zones of inhibition around each cup were measured, with the aid of calipers, after the incubation period. The regression coefficient (b) of a plot of the mean diameters, given by the 4 replicates of each of the 5 standard solutions, versus the logarithm of the ampicillin concentration in those solutions was calculated for each plate. The unknown concentrations of ampicillin in the plasma samples used on that plate were then determined by means of Eq. 4.1,

$$X = \frac{(Y - \bar{Y}) + b\bar{X}}{b} \qquad \text{Eq. 4.1}$$

where X = log concentration, Y = zone diameter and $\bar{X}$ and $\bar{Y}$ are the

mean values of these parameters.

4.3 Results

All zero time plasma samples yielded, without any exception, no detectable activity against the test organism. This is in agreement with the findings of Macleod et al (1974). The mean concentrations of ampicillin in the plasma samples that were taken from the 6 rabbits at various times after oral administration of ampicillin trihydrate suspensions are given in Table 4.2. Plots of the mean concentrations versus time are shown in Fig. 4.1.

The values of the 3 bioavailability parameters, i.e. AUC_o^8, PC and PT, are given in Table 4.3. The AUC_o^8 was calculated by using the trapezoidal rule.

The statistical analyses of the bioavailability parameters were carried out using the methods given in Chapter 2 of this Section. The results of these analyses, which are summarised in Table 4.4, show that there are no significant differences between the AUC_o^8 values or the PT values ($P > 0.05$) and that only the PC for formulation D differs significantly from those of B, F and E ($p < 0.05$).

Rabbit No. 5 died before carrying out the last experiment for formulation B in the time period 6. Therefore the missing values for the three bioavailability parameters (PC, PT and AUC_o^8) were calculated according to equation 2.1 described in Chapter 2 of this Section.

Table 4.2 Mean plasma concentrations of ampicillin ($\mu g/cm^3$) following oral administration of 50 mg/kg body weight of ampicillin trihydrate, as a single dose in different formulations, to 6 rabbits.

Time(hr) / formul^n(a)	½	1	1.5	2	3	4	6	8
A	2.63	2.59	2.42	2.45	3.65	3.57	2.32	1.31
B	3.35	2.94	2.79	2.99	3.14	2.83	1.49	0.55
C	4.52	4.77	4.01	3.02	1.60	0.74	0.21	-
D	4.40	5.86	6.05	4.90	3.27	1.72	0.54	-
E	2.50	3.26	2.39	2.00	2.10	1.98	0.98	0.73
F	2.50	3.20	2.53	2.57	2.37	2.48	1.40	0.66

Key (a) the formulations are as specified in Table 4.1

Table 4.3 Mean peak plasma concentrations (PC), peak times (PT) and area under the curve (AUC_o^8) of ampicillin following oral administration of 50 mg/kg body weight of ampicillin trihydrate, as a single dose in different formulations, to 6 rabbits.

Parameter / Formul^n (a)	A	B	C	D	E	F
PC ($\mu g/cm^3$)	4.9	3.8	5.2	6.4	3.5	3.7
PT (hr)	2.1	1.3	1.0	1.2	1.9	1.8
AUC_o^8 ($\mu g.hr/cm^3$)	20.1	17.4	12.0	18.6	13.2	15.3
η_{app} (b)	17.5	64	0.695	2.32	150	140

Key: (a) The formulations are as specified in Table 4.1

(b) η_{app}, apparent viscosity, from Table 1.2,Section 2.

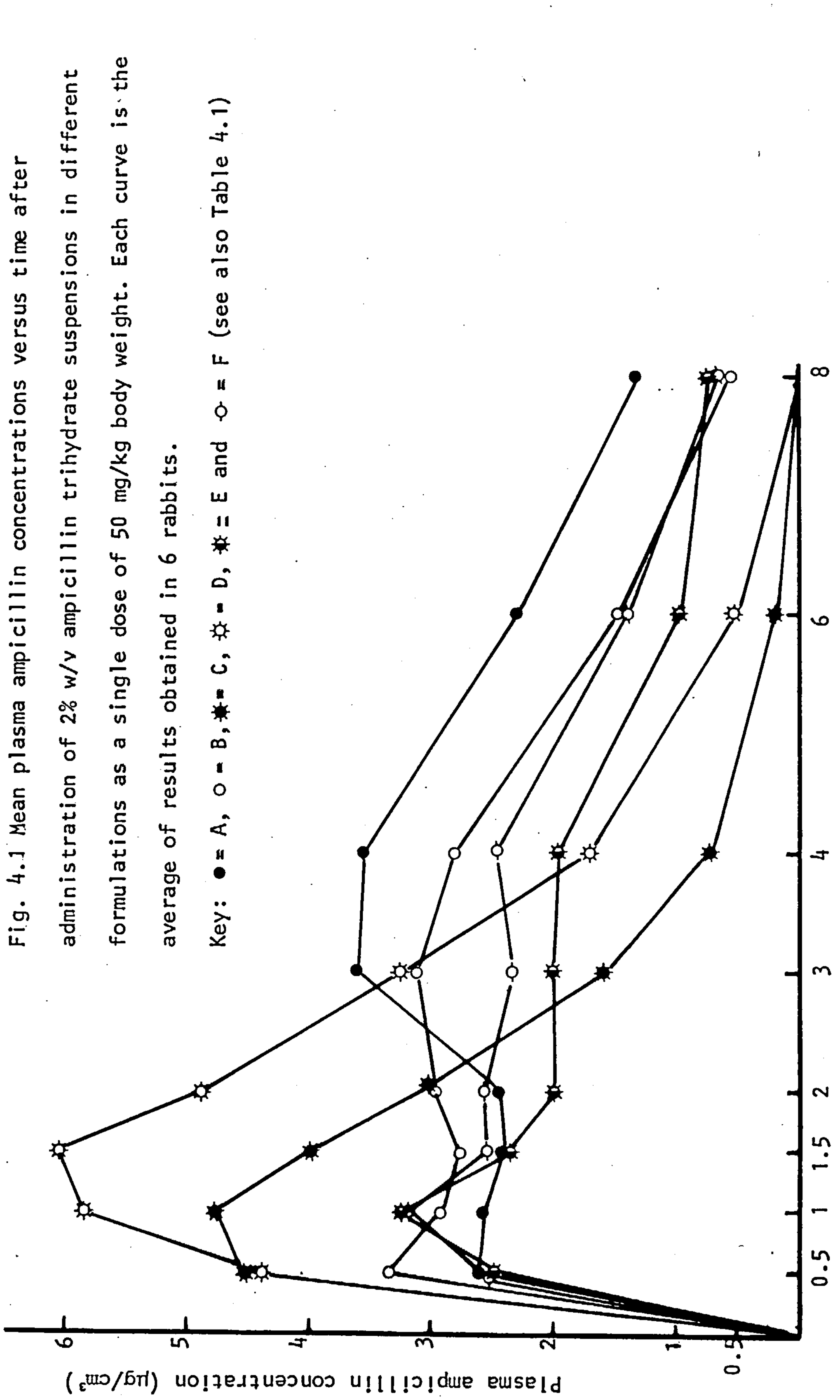


Fig. 4.1 Mean plasma ampicillin concentrations versus time after administration of 2% w/v ampicillin trihydrate suspensions in different formulations as a single dose of 50 mg/kg body weight. Each curve is the average of results obtained in 6 rabbits.

Key: ● = A, ○ = B, ✱ = C, ☼ = D, ✱ = E and ☼ = F (see also Table 4.1)

Table 4.4 Results of the analysis of variance and Duncan's multiple range test on the values obtained for PT (hr), PC ($\mu g/cm^3$) and AUC_o^8 ($\mu g.hr/cm^3$) of ampicillin after administration of 50 mg/kg body weight as a single dose of ampicillin trihydrate to rabbits in a 6 x 6 latin square.

PT	Formulation(a)	C	D	B	F	E	A
(hr)	Mean (b)	1.00	1.20	1.3	1.80	1.90	2.10
PC	Formulation(a)	E	F	B	A	C	D
($\mu g/cm^3$)	Mean (b)	3.50	3.7	3.8	4.9	5.2	6.4
AUC_o^8	Formulation(a)	C	E	F	B	D	A
($\mu g.hr/cm^3$)	Mean (b)	12.0	13.2	15.3	17.4	18.6	20.1

Key: (a) The formulations are as specified in Table 4.1.

(b) Any two means not underlined by the same line are significantly different ($p<0.05$). Any two means underlined by the same line are not significantly different ($p>0.05$).

4.4 Discussion

The results obtained in this study, although not significantly different, $p > 0.05$, showed that the AUC_o^8 of ampicillin administered as a simple aqueous suspension (C) was 60%, 64%, 68%, 78% and 90% of A, D, B, F and E respectively. In other words, if it is considered that the simple oily suspension (A) is 100% bioavailable, then the bioavailability of (C) is 60% of (A). Inclusion of 30% w/v sucrose in the aqueous suspension (D) enhanced the extent of absorption to a degree which is close to that of formulation A.

A variety of factors could be responsible for the slight enhancements in the extent of absorption of ampicillin, as mentioned in Chapter 1 in this Section. The most likely explanation is, in fact, decrease in the GER brought about by oil or osmotic pressure (see Chapter 2, Section 1), because the inclusion of 30% sucrose in the aqueous suspension (formulation D) enhanced the extent of absorption of ampicillin, as reflected by the AUC_o^8, from 12 to 18.6 $\mu g.hr/cm^3$, which is close to the AUC_o^8 of 20.1 $\mu g.hr/cm^3$ for formulation A(Table 4.3).

Since ampicillin is an amphoteric compound with an isoelectric point of 4.95, it follows that its solubility will be greater in the gastric fluid than in the mildly acidic intestinal fluid, which has a pH closer to the isoelectric point. Thus, a longer gastric residence time of ampicillin would improve dissolution and enhance bioavailability. In addition,Kirby and Kind (1967) indicated that appreciable absorption of ampicillin occurred from the stomach and Swahn (1976) reported that the absorption of radiolabelled ampicillin from the stomach is up to 30% of the total amount absorbed. Therefore, if GER is decreased the degree of absorption from the stomach might be increased so enhancing the overall extent of absorption of ampicillin.

If the extent of absorption of ampicillin is increased by a reduction in the GER then one would expect this extent to be reduced by an increase in GER as found by Ali and Farouk (1980) who studied the effect of Sudanese diet on the bioavailability of ampicillin. They indicated that the reduced extent of absorption and PC could be attributed to increases in the GER and the total GI motility caused by the Sudanese diet, which is rich in bran and fibrous substances that are known to accelerate gastric emptying and might also increase the GI motility. They commented"the nett result of this effect will be a short drug residence time and consequently less absorption of the administered drug. Moreover, this effect will vary according to the time of the meals when the drug is administered. Consequently, one would expect maximum absorption of the drug if sufficient time is given for the drug absorption before such food is taken". They suggested that when gastric emptying is delayed, ampicillin will stay in the GI tract longer, and hence more complete absorption will occur. The results obtained in the present study are in good agreement with these findings and suggestion.

If the enhancement in the extent of ampicillin absorption from formulations A and D compared to that from C can be explained solely on the basis of the decrease in GER, brought about by oil or by sucrose, it leads to the conclusion that the effects of oil on gastric secretion, formation of bile salt-mixed micelles, stimulation of the lymph flow and the effect of viscosity of the formulation, (Table 4.3), (see Chapter 1 in this Section) are unlikely explanations of the results obtained in this study. It is suggested, therefore, that delay in the GER is the most likely explanation of the enhancement obtained in the extentof ampicillin absorption when the drug is administered in oily formulations and formulations possessing high osmotic pressure.

The oily formulation E, whose vehicle consists of 1.25% w/v Cab-o-sil plus 30% w/v sucrose dispersed in FCO, and formulation F, which corresponds to that described by Stephens and Su (1975), allowed a greater extent of ampicillin absorption than the simple aqueous suspension (formulation C) but provided a lower extent than A,D or B. This latter rank order relationship, i.e. extent of absorption from A, D and B > extent of absorption from F and E, is paralleled by the results obtained in the in vitro dissolution rate studies that are described later in this thesis (Chapter 1, Section 4). It is possible, therefore, that the in vivo results can be explained to some extent on the basis of differences in the rates of release of drug from various formulations.

In the case of the oily formulation E, it may be suggested that the possible adsorption of ampicillin on to the Cab-o-sil may interfere with the release of the drug and so reduce the bioavailability. However, this suggestion is unlikely because Poole et al (1968) detected no change in the bioavailability of ampicillin when 0.99% Cab-o-sil was included in their aqueous suspension.

It should be pointed out that, unlike salicylate, the inclusion of 30% w/v sucrose in the oil, i.e. formulation B, did not enhance the extent of ampicillin absorption but it did enhance the absorption from the aqueous formulation D. It is suggested, therefore, that sucrose enhances the absorption of ampicillin from the aqueous vehicle by virtue of its delaying effect on the GER and has no additional effect on the GER over that caused by the oil itself. This suggestion is supported by the fact that AUC values of formulations A, B and D are close to each other (see Table 4.3). Furthermore, the lack of any enhancement effect by sucrose on the extent of ampicillin absorption in the oily vehicle may also be related to the fact that the partition coefficient of

ampicillin between oil and 0.1 mole/dm³ HCl is less than unity, i.e. 0.052, (see Chapter 2, Section 4) and therefore the role of the osmotic effect of sucrose in minimising the uptake of water by the GI membrane, and hence maintaining a large volume of available water in the GI tract, is not as important as in the case of drugs with oil: HCl partition coefficients of more than unity, i.e. salicylate. This finding, therefore, is further evidence for the suggestion made to explain the enhancing effect of sucrose on salicylate absorption when administered in the oil (see Chapter 2 in this Section).

Table 4.4 shows that no significant difference was detected in the PT values for all the formulations, $p>0.05$. This suggests that ampicillin is absorbed essentially at the same rate from the oily and aqueous formulations. Ampicillin is more hydrophilic than salicylate (see Chapter 2, Section 4), and hence one does not expect the oil to be a reservoir for this drug. However, the trend toward a slower absorption from the oily formulations is likely because of the delay in the GER by the oil, which is more pronounced than that caused by the osmotic pressure exerted by aqueous formulation D.

Comparison of the PCs of the aqueous suspensions (C and D) with those of the oily ones, Table 4.4 and Fig. 4.1, suggests that higher concentrations are provided by the former systems. However, only the aqueous one containing sucrose (D) gave a significantly different PC value from those obtained with the oily B, E and F formulations ($p<0.05$) and the PC provided by formulation A, the simple oily suspension, did not differ significantly from either of the aqueous formulations C and D ($p>0.05$).

It should be pointed out that when comparison between the plasma concentration versus time curves of ampicillin given by the oily and aqueous systems is made, Fig. 4.1 and Table 4.2, it is quite clear

that all the oily formulations still maintain a measurable plasma concentration at 8 hr post-administration whilst the aqueous ones gave zero concentration at that time. In fact, some individual rabbits gave zero plasma concentration as early as the 4-6 hr samples. The prolonged plasma concentrations of ampicillin, that are obtained when oily vehicles are used, may arise because oil stimulates the evacuation of bile from the gall bladder (see parts 1.4.2 b and d in Section 1) and it is well known that ampicillin is rapidly excreted in the bile in an active form (Stewart and Harrison, 1961; Harrison and Stewart, 1961; Acred et al, 1962; Ayliffe and Davies, 1965; Lund et al, 1976) with a low susceptibility to the inactivating mechanism within the liver (Tuano et al, 1966; Kirby and Kind, 1967). Enhancement of biliary recycling by the oil would therefore lead to reabsorption of ampicillin and a prolongation of blood levels (see part 1.4.1 c, Section 1). In fact, Ritschel (1980a) stated that "drugs entering the bile must be considered as drugs administered perorally. Upon emptying of the bile into the duodenum, the drugs may be re-absorbed by one of the absorption mechanisms into the portal circulation and returned to the liver from whence they are re-excreted into bile". Therefore, enterohepatic recycling is the most likely explanation of the occurrence of the multiphasic blood level curves, with two peaks, that were given by the oily formulations (see part 1.4.1 c, Section 1). In some cases the second peak was regarded as the peak plasma concentration, as in formulation A, since it is higher than the first one. An alternative explanation of this periodicity is that it could be attributed to the sequential absorption of the drug, first from the stomach and then from the small intestine after gastric emptying had occurred. However, this latter explanation seems to be unlikely since the aqueous formulation D, which should also delay GER, because of its sucrose

content, did not show this phenomena (i.e. a multiphasic blood level curve). Thus, enterohepatic recycling seems to be the most likely explanation of this effect.

The first peak occurred during the first hr and the second one after 3 hr with all the oily formulations and in all the rabbits without any exception. None of the aqueous formulations showed this phenomenon.

Stewart and Harrison (1961) and Harrison and Stewart (1961) reported the rapid and immediate excretion of ampicillin in the bile. The liver was found to be capable of clearing up to 7% of a large dose from the blood in 1 hr producing a concentration in the bile, which may be 40 times higher than the peak plasma concentration, e.g. 20-160 $\mu g/cm^3$ in the bile 1 hr after dosing. This concentration increased sharply during the next 2 hr, and peak values of 160 to 440 $\mu g/cm^3$ were obtained between 2.5 - 7.5 hr. Re-absorption produced plasma increments of about 1 $\mu g/cm^3$, occasionally more. The first phase of absorption of the orally administered drug into the plasma occurred between 1 and 3 hr; the second phase, i.e. reabsorption, added small increments to the plasma concentration during this time and up to 6 hr. These results of Stewart and Harrison agree with those obtained in this study. Ayliffe and Davies (1965) also reported that, in patients with normal biliary tracts, levels of up to 48-times those in normal serum were obtained in gall bladder bile within 4 hrs. These findings provide an explanation of the results of Acred et al (1962), who showed that the concentration of ampicillin fell off more rapidly in the serum than in liver and kidney, because the presence of bile and urine would favour concentration of ampicillin in these two organs.

Williams et al (1965) have provided an excellent discussion of the factors affecting the enterohepatic circulation of drugs and the consequences of the recycling process and the therapeutic value of

such circulation in the treatment of biliary and intestinal tract infections has been pointed out (Harvey, 1975; Martindale, 1977b; Sjoqvist et al, 1980).

From the above points and references it can be concluded that prolonged plasma concentrations may be attributed to enterohepatic recycling. This conclusion is supported by Kirby and Kind (1967) and by a comparison of the results obtained with formulations A and D.

Finally, differences in the renal clearance will obviously influence the blood levels of ampicillin and the duration of these levels. The variation introduced by this effect is minimised by the use of cross-over tests. Reduced renal clearance was found to produce higher and later peak plasma levels than normal renal function (Kirby and Kind, 1967; Hultberg and Backelin, 1972). Therefore, in those instances where the normal renal function has not yet been developed, as in the newborn (see part 1.4.3 d, Section 1), the higher and longer duration of ampicillin blood levels would be expected to be dangerous and even lethal. Evidence supporting this effect has been found in this study, because in preliminary work with rabbits weighing less than 2 kg, a dose of 50 mg/kg body weight was found to be a lethal dose. The rabbits died after a period of 2-5 days after dosing. However, a dose of 100 mg/kg body weight was not lethal in rabbits weighing more than 2.5 kg. Subsequent studies were carried out with rabbits weighing more than 2 kg and a dose of 50 mg/kg body weight was used. It is recommended that this point should be borne in mind when designing bioavailability tests for ampicillin preparations.

In conclusion, delay in the GER, caused by the effect of oil or the osmotic effect of sucrose, produced a slight enhancement in the extent of ampicillin absorption. This enhancement was not statistically significant ($p > 0.05$) but was close to the borderline of significance.

In addition, although ampicillin appears to be absorbed at essentially the same rate from both aqueous and oily formulations, the latter are likely to produce prolonged plasma concentrations of ampicillin because of the effects of enterohepatic recycling.

SECTION 4

IN VITRO STUDIES

CHAPTER 1

DISSOLUTION RATE STUDIES

1.1 Introduction

Details of the usefulness of *in vitro* release studies are given in parts 1.2.2 and 1.2.3 of Section 1. As already mentioned there, the development and use of *in vitro* models to discriminate between different formulations and to predict the availability of drugs in particular dosage forms is important when physiological functions play no significant part in regard to this aspect. As no apparatus or procedure can exactly duplicate *in vivo* conditions, all dissolution studies are relative, and the most important considerations are ones of reproducibility, practicality and reasonableness. However, in spite of this limitation, *in vitro* models still serve as a secondary standards for the detection of the differences in the release of drugs from different dosage forms, and the effect of different pharmaceutical additives.

In vitro studies on the release of drugs from suspension formulations have included methods involving the use of either flask - stirrer or dialysis methods. The use of a dialysis membrane has been advocated by several workers as a device for obtaining the solute concentration without disturbing the dissolution process. Depending on the apparatus used it is possible to carry out dissolution studies under sink conditions. The choice of the membrane is important, for it must have a short equilibrium time and adequate physical strength and retain solid particles. Obviously, the rate at which solute appears on the distal side of the membrane should not be a function of the dialysis rate but of the dissolution rate. In other words, within such an *in vitro* model system dissolution must be the rate-limiting step

(Swarbrick, 1970). A variety of dialysis methods have been developed to study the *in vitro* release rate of drugs from tablets and capsules (Marlowe and Shangraw, 1967; Barzilay and Hersey, 1968), from suspensions (Marty and Hersey, 1975a and b; Shah and Sheth, 1976; Barzegar-Jalali and Richards, 1979a) and from solutions (Lamy, 1969; Bachynsky et al, 1976).

The flask-stirrer method was introduced by Poole (1969). It has been used by Bates et al (1969) to obtain an *in vitro - in vivo* correlation for salicylamide dosage forms including a commercial suspension of the drug and Bates et al (1973) to compare the dissolution rates of nitrofurantoin from a commercial suspension and a tablet dosage form.

Although the sensitivity of the flask-stirrer method has been proved to be adequate in detecting differences in the effects of suspending agents on the dissolution rate of nitrofurantoin suspensions, this was not the case in another study on aspirin suspensions, where a more sensitive dialysis method had to be used (Barzegar-Jalali and Richards, 1979a and 1980). In addition, a dialysis method was used to study the effect of macromolecules and different gums on the release of salicylic acid and sodium salicylate, respectively, from aqueous solutions (Lamy, 1969; Bachynsky et al, 1976).

The work described in this chapter is concerned with release studies on the 3 drugs involved in this thesis, i.e., sodium salicylate, nitrofurantoin and ampicillin, using flask-stirrer and dialysis methods, in an attempt to detect the possible effects of the different pharmaceutical additives, which are involved in the oily vehicles patented by Stephens and Su (1975) and Lin and Pramoda (1978), on the release characteristics that could be masked by the predominant effect of the oil *in vivo*.

1.2 Experimental

1.2.1 Material

Details of the sources of the materials and methods of preparation of the suspenions are given in Sections 2 and 3.

1.2.2 Methods

(a) Dialysis Method

In addition to a 4% w/v suspension of sodium salicylate in FCO alone (A) and a 4% w/v solution of the salicylate in distilled water (B), the release studies were carried out on sodium salicylate suspensions of the same concentration in the following types of vehicles.

Type 1 vehicles

Aluminium stearate (50:50 mixture of mono and di-stearates) in the following concentrations in FCO:

A = 0.5% w/v

B = 1 % w/v

C = 1.5% w/v

D = 2 % w/v

E = 2.5% w/v

F = 3 % w/v

G = 3.5% w/v

H = 4 % w/v

I = 5 % w/v

Type 2 vehicles

(Related to the formulation of Stephens and Su (1975)).

A = 0.7% w/v lecithin in FCO

B = 0.35% w/v hydrogenated castor oil in FCO

C = 0.5% w/v aluminium stearate + 0.7% w/v lecithin + 0.35% w/v

hydrogenated castor oil + 20% w/v sucrose in FCO.

D = 0.5% w/v aluminium stearate + 0.35% w/v hydrogenated castor oil + 20% w/v sucrose in FCO.

Type 3 vehicles

(Related to the vehicle of Lin and Pramoda (1978)).

A = 20% w/v sucrose in FCO

B = 0.3% w/v Cab-o-sil + 20% w/v sucrose in FCO

C = 0.5% w/v Cab-o-sil + 20% w/v sucrose in FCO

D = 1% w/v Cab-o-sil + 20% w/v sucrose in FCO

The dialysis method and apparatus used in this study was based on that described by Barzegar-Jalali and Richards (1979a) with minor modifications. One end of a 25 cm length of Visking dialysis tubing (Scientific Instrument Centre, Ltd.), having an inflated diameter of 2.14 cm, was tied off after the tubing had been soaked in HCl (0.1 mole/dm^3) for at least 12 hr. Seventy glass beads with an approximate diameter of 3 mm were placed in the tube. These beads regulate the oscillations of the dialysis sac and markedly improved the reproducibility of the results obtained for aqueous suspensions (Barzegar-Jalali and Richards, 1979a). Five cm^3 of a suspension were poured into the sac and that part of the sac above the level of its contents was flattened between the fingers. The sac was then suspended through the central neck of a 2 dm^3 two-necked round-bottomed flask, which contained 1495 cm^3 of 0.1 mole/dm^3 HCl, and secured by a glass stopper in such a way that the surface of the contents of the sac was 1 cm below the surface of the dissolution medium. Use of these volumes allowed sink conditions to be maintained because the solubility of sodium salicylate in 0.1 mole/dm^3 HCl is 306.1 mg/100 cm^3 (see Chapter 2, this Section). A thermometer and a glass tube connected to a flexible plastic tube were inserted through a rubber stopper in the side neck of the flask and into

the dialysis medium. The plastic tubing facilitated sampling whilst the flask was being shaken. The whole assembly was clamped in a shaking water bath (Gallenkamp) maintained at 37°C ± 0.1°C and adjusted to provide an oscillation frequency of 120 ± 2 cycles/min. At this frequency not only was the dialysis medium well agitated but also the sac was oscillated in a constant manner, thus ensuring good mixing on either side of the membrane and preliminary experiments showed that this frequency was low enough to discriminate between the release rates of the different formulations. A diagram of the dialysis apparatus is given in Fig. 1.1.

Five cm^3 samples were taken from the flask at various times and immediately replaced by the same volumes of 0.1 mole/dm^3 HCl. The samples were filtered through a Millipore filter assembly (0.45 μm pore size). The absorbance of the solution was determined using an SP 500 Unicam spectrophotometer at 300.5 nm with 0.1 mole/dm^3 HCl as the reference solution. The corresponding concentration was derived from a calibration curve using equation 1.1, which is described in Chapter 1 of the previous Section (i.e. Eq. 1.2). The concentrations of the standard solutions used to construct this calibration curve and their corresponding absorbances at 300.5 nm are given in Table 1.1. The Beer-Lambert law was obeyed over the range of concentrations indicated in the table.

Fig. 1.1 Dialysis apparatus used for the release studies on sodium salicylate from aqueous solution and oily suspensions.

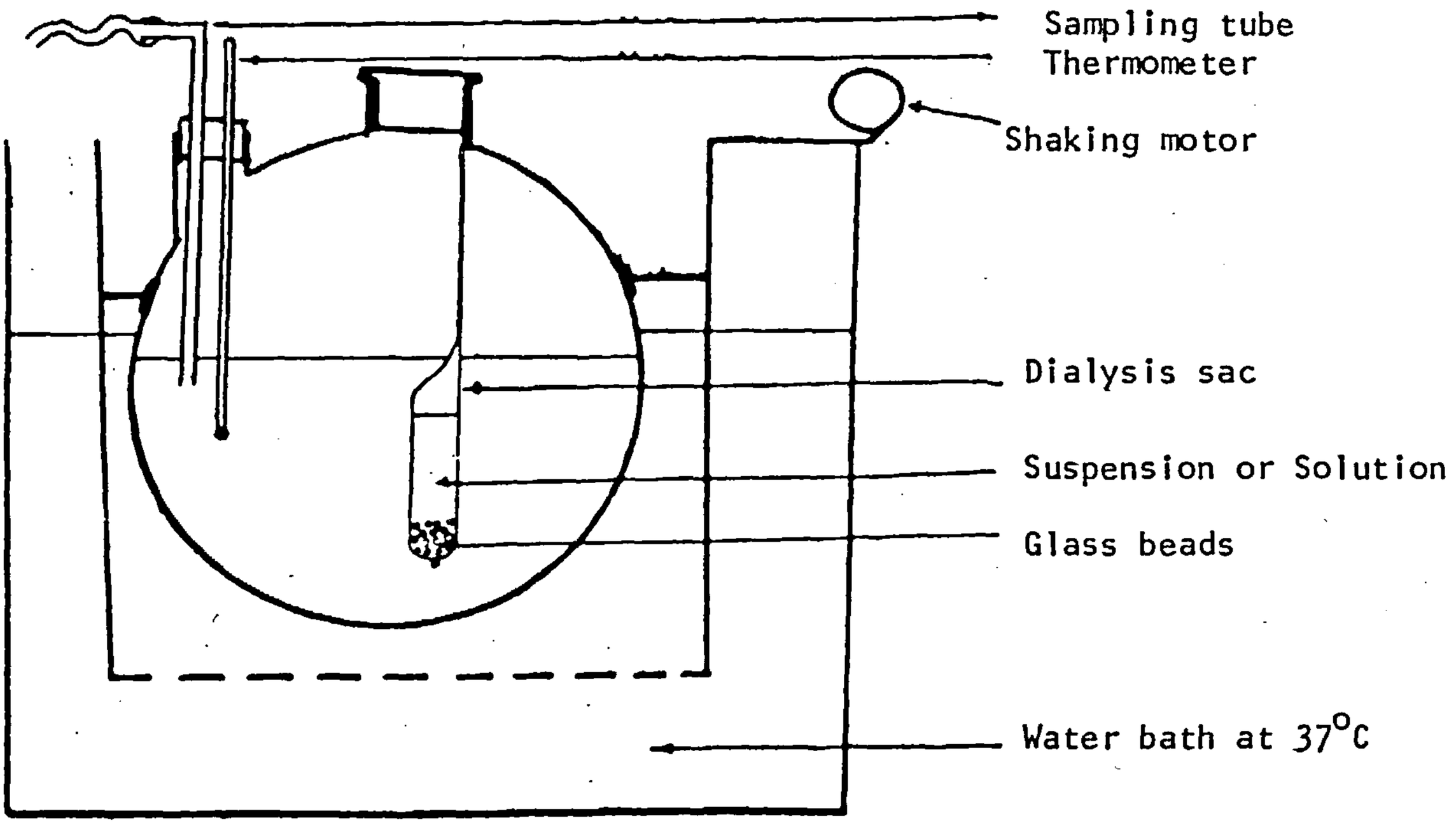


Table 1.1 Data for calibration curve of sodium salicylate in 0.1 mole/dm³ HCl at 300.5 nm.

Concentration (X) (mg/100 cm³)	Absorbance (Y)
0.5	0.117
1	0.225
2	0.454
3	0.677
4	0.905
5	1.120

$$X = \frac{Y - 0.006}{0.2236} \qquad \text{Eq. 1.1}$$

(b) Flask-stirrer method

(i) Nitrofurantoin suspensions

The suspensions A-H, described in Chapter 3 of the previous Section, were used in this in vitro study. The method was based on the apparatus described by Poole (1969) and used by Barzegar-Jalali and Richards (1979a). A 2 dm³ wide-mouth, round-bottomed flask, with a lid comprising of four side necks and one central neck, was placed in a water-bath maintained at 37 ± 0.1 °C. A 2-bladed, 8.1 cm diameter glass stirrer was placed through the central neck and located in a standard position relative to the bottom of the flask, i.e. 5 cm from the bottom of the flask, and connected to an electric motor (Citenco Ltd.), which rotated the stirrer in a counter-clockwise direction at a speed of 60 ± 2 r.p.m. A suitable thermometer (0-50°C) and a plastic cannula for sampling were placed at a constant height, angle and position into the dissolution medium through the side necks.

The other side neck was used to introduce the dissolution medium (1480 cm^3 of 0.1 mole/dm^3 HCl solution) and sample suspension. A diagram of the apparatus is shown in Fig. 1.2.

While the stirrer was in motion 10 cm^3 of an overnight aged suspensions were injected through the side neck from a 10 cm^3 graduated syringe. The latter was washed out with 10 cm^3 of 0.1 mole/dm^3 HCl and the washings were also added to the flask. Using 10 cm^3 of a 0.1% w/v nitrofurantoin suspension in 1490 cm^3 of dissolution medium provided sink conditions for the drug, because its solubility in 0.1 mole/dm^3 HCl at 37^{o}C is 15.59 mg/100 cm^3 (see next Chapter).

Exposure of the nitrofurantoin solutions, i.e. the standard solutions and the solution in the dissolution flask, to light was minimised as far as possible by wrapping the containers with aluminium foil or with black plastic film. Samples of the dissolution medium were obtained at various times in a similar manner to that used in the previous dialysis method. The absorbance of each solution at 369 nm was determined using 0.1 mole/dm^3 HCl as the reference solution. Table 1.2 shows the concentrations of standard solutions of nitrofurantoin in 0.1 mole/dm^3 HCl and their absorbance value at 369 nm that were used to construct the necessary calibration curve. The same equation that is described in Chapter 1 of the previous Section (i.e. Eq. 1.2) was used to calculate the concentrations of nitrofurantoin in the different samples.

Table 1.2 Data for calibration curve of nitrofurantoin in 0.1 mole/dm³ HCl at 369 nm

Concentration (X) (mg/100 cm³)	Absorbance (Y)
0.1	0.08
0.2	0.15
0.3	0.22
0.4	0.295
0.6	0.44
0.8	0.585
1	0.73

$$X = \frac{Y - 0.005}{0.7242} \qquad \text{Eq. 1.2}$$

(ii) Ampicillin suspensions

The suspensions A-F, described in Chapter 4 of the previous Section, were studied. The apparatus was the same as that used for nitrofurantoin except for the volume of the dissolution medium, which was 200 cm³, and the speed of the stirrer was 60 ± 2 r.p.m. 10 cm³ of 2% w/v freshly prepared suspensions of ampicillin trihydrate were injected through the side neck of the dissolution flask from a 10 cm³ graduated syringe. The latter was washed out with 5 cm³ of 0.1 mole/dm³ HCl and the washings were also added to the flask. Sink conditions prevailed because the solubility of ampicillin in 0.1 mole/dm³HCl at 37°C is more than 2% w/v as mentioned in Chapter 4 of the previous Section. 2 cm³ samples were removed at specified time intervals and each sample was replaced immediately by 2 cm³ of 0.1 mole/dm³ HCl. The samples were filtered through a Millipore filter and the ampicillin content of each was determined by the method of Smith et al (1967) with minor modifications as follows:

1 cm³ was diluted to 50 cm³ with a previously prepared pH 5.2 buffer solution containing copper sulphate. 10 cm³ of this solution

were transferred to a calibrated test tube, which was lightly stoppered and placed in a thermostatically controlled bath at 75°C. After exactly 30 min the tube was removed from the bath and rapidly cooled in room temperature. The absorbances of the solutions were then measured at 320 nm using unheated buffered ampicillin solution as the blank. The concentrations were derived from a calibration curve prepared by carrying out the above procedure on known dilutions of a standard ampicillin preparation. Table 1.3 shows the data used in obtaining such a curve. Eq. 1.2 in Chapter 1 of the previous Section was again used to calculate the concentrations of the unknown samples.

The buffer solution was prepared by mixing 464 cm^3 of 0.1 mole/dm^3 citric acid solution and 536 cm^3 of 0.2 mole/dm^3 disodium hydrogen phosphate solution. The pH was adjusted, if necessary, to 5.2 $\pm$ 0.05 with either of the above mentioned solutions. To 15 cm^3 of a 0.393 w/v copper sulphate pentahydrate solution, the mixed buffer at pH 5.2 was added to a volume of 1 dm^3. One cm^3 of this solution contains 15 μg of copper.

Table 1.3 Data for calibration curve of ampicillin trihydrate in 0.1 mole/dm^3 HCl at 320 nm.

Concentration mg/100 cm^3 0.1 mole/dm^3 HCl	Concentration mg/100 cm^3 pH 5.2 buffer (X)	Absorbance (Y)
12.5	0.25	0.03
25	0.5	0.062
50	1.0	0.124
75	1.5	0.186
100	2.0	0.255

$$X = \frac{Y + 0.00258}{0.1276} \qquad \text{Eq. 1.3}$$

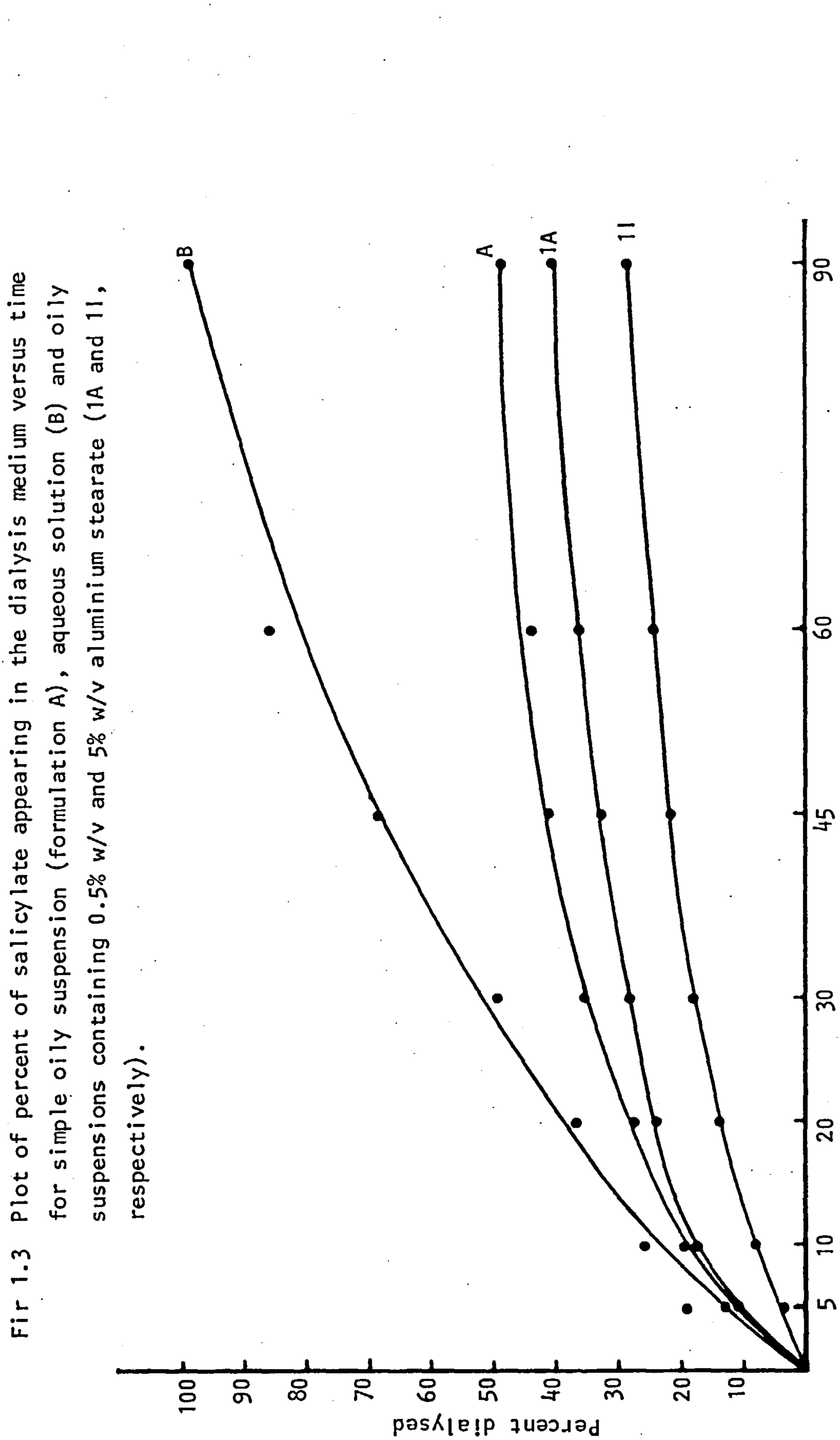
1.3 Results

(a) From dialysis method

The amount of salicylate released, expressed as a percentage of the total amount originally added to the system, was calculated from the drug concentration in each sample and plotted against the sampling time to give a dialysis, or release rate curve. The mean values of these percentages for each formulation are shown in Table 1.4. Each value is the mean obtained from 3 experiments. The release rate curves for these values are shown in Fig. 1.3-1.5. Fig. 1.3 shows the lower and higher concentrations of aluminium stearate, i.e. 0.5% w/v and 5% w/v. All the other concentrations lie between these two curves but have been omitted from the figure for the sake of clarity.

The time required for 30% and 50% of the salicylate to appear in solution outside the dialysis sac ($t_{30\%}$ and $t_{50\%}$) were calculated from the individual release rate curves for each formulation and were used as indices for estimation of the release rate. Analysis of variance and Duncan's test were carried out to distinguish the differences between the mean $^{t}30\%$ values. Details of the analysis were described in Chapter 2 of the previous Section. The results of the analysis can be summarised as shown in Table 1.5 where any two means not underlined by the same line are significantly different ($p<0.05$ or $p<01$) and any two means underlined by the same line are not significantly different.

The results given in Table 1.5 show that the sensitivity of the method in discriminating between the different formulations is not as good as might be expected from a simple comparison of the mean values of $^{t}30\%$. This reduction in sensitivity is brought about by the relatively low reproducibility of the release of salicylate from some of the systems. It is considered that this low reproducibility is related to the nature of the suspensions that are being tested and



Fir 1.3 Plot of percent of salicylate appearing in the dialysis medium versus time for simple oily suspension (formulation A), aqueous solution (B) and oily suspensions containing 0.5% w/v and 5% w/v aluminium stearate (1A and 11, respectively).

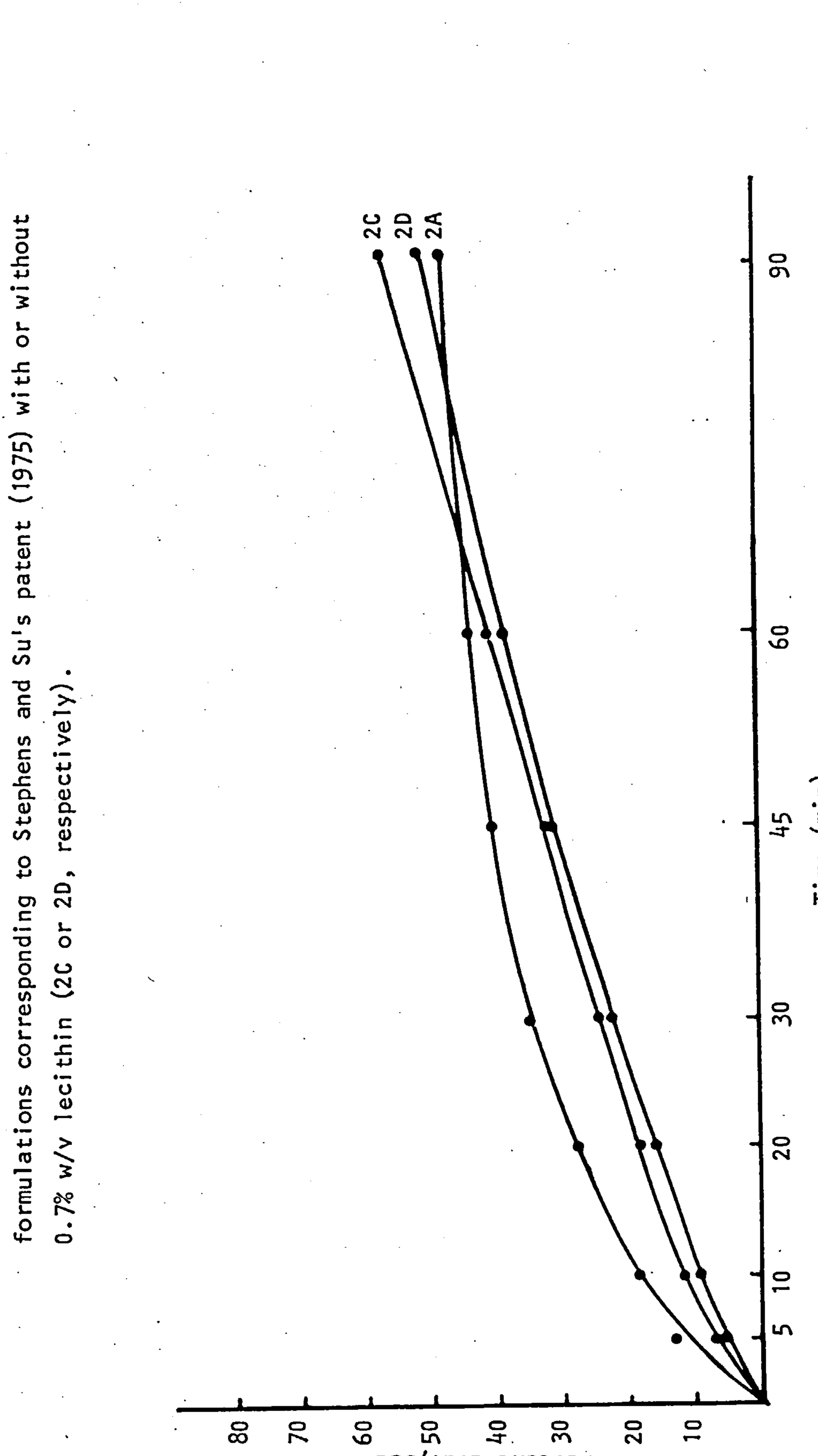


Fig 1.4 Plot of percent of salicylate appearing in the dialysis medium versus time for an oily suspension containing 0.7% w/v lecithin in FCO (2A) and formulations corresponding to Stephens and Su's patent (1975) with or without 0.7% w/v lecithin (2C or 2D, respectively).

Fig 1.5 Plot of percent of salicylate appearing in the dialysis medium versus time for oily suspensions containing 20% w/v sucrose plus 0.3% w/v or 1% w/v Cab-o-sil in FCO (3B or 3D, respectively).

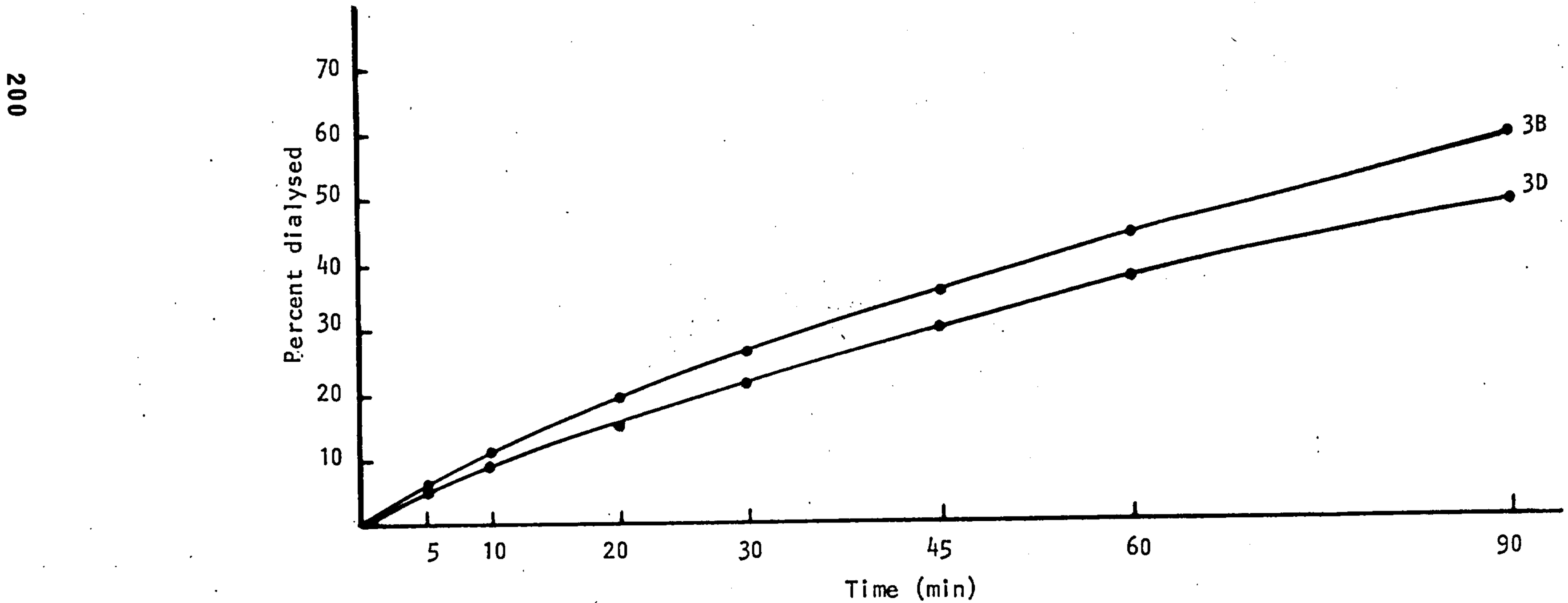


Table 1.4 Percent[(a)] salicylate released at various times from different formulations using the dialysis method.

Formul[n] (b) \ Time (min)	5	10	20	30	45	60	90
A	13.2	19.2	27.8	35.2	40.6	43.9	48.6
B	19.2	25.6	36.8	49.3	68.4	85.8	98.5
1A	10.8	17.4	24.3	28.1	32.8	36.0	40.8
1B	10.4	17.0	22.8	27.5	32.2	35.8	40.4
1C	9.0	14.4	20.6	25.8	29.0	31.9	36.8
1D	8.4	13.7	19.4	23.6	27.9	32.9	36.0
1E	7.5	12.7	18.7	23.9	28.9	31.7	35.7
1F	6.1	10.8	17.3	22.3	27.1	30.4	34.9
1G	5.0	9.0	15.2	19.9	24.1	26.4	30.1
1H	5.5	8.8	14.6	18.1	21.2	23.9	28.7
1I	3.4	8.2	14.0	17.8	21.6	24.1	28.1
2A	13.0	18.8	27.4	35.0	40.0	43.4	47.7
2B	11.8	19.5	28.0	34.7	39.2	42.2	46.9
2C	5.4	9.3	15.9	22.3	31.9	40.9	56.8
2D	7.3	11.9	18.3	24.4	32.2	38.5	51.0
3A	7.9	11.7	19.2	26.6	34.7	43.3	56.3
3B	6.9	11.0	19.4	26.6	35.9	44.4	58.9
3C	6.6	10.3	17.6	24.5	34.1	43.5	55.6
3D	5.6	9.3	15.8	21.5	30.1	38.1	48.5

(a) Each value is the average of the results from three experiments.

(b) Key to Formulations: A = simple suspension in FCO, B = aqueous solution, Type 1 vehicles (A-I) contain various concentrations of aluminium stearate in FCO, Type 2 (A-D) and Type 3 (A-D) vehicles are based on those of Stephens and Su (1975) and Lin and Pramoda (1978), respectively; further details are given on pages 188 and 189.

Table 1.5 Summary of the results of the statistical analysis of $^{t}30\%$ values for formulations containing sodium salicylate

Formulation (a)	B	A	2A	2B	1A	3B	3A	1B	3C	2D	2C	3D	1C	1D	1E	1F	1G	1H	1I
Mean values of $^{t}30\%$ in rank order p = 0.01	14.3	22.2	22.7	22.9	34.0	35.3	36.7	37.1	38.6	41.1	42.2	44.4	45.3	50.7	51.5	58.3	89.2	99.7	110
P = 0.05	B	A	2A	2B	1A	3B	3A	1B	3C	2D	2C	3D	1C	1D	1E	1F	1G	1H	1I

(a) The formulations are as specified on pages 188 and 189.

the complexity of the mechanisms that will influence the overall release process. These mechanisms are described in Section 1.4a of this Chapter.

A cumulative correction to account for the previously removed samples was not made when determining the percentage of drug released because, as can be seen from the following calculations, which make use of data obtained for the simple suspension in the oil (A), the corrected and uncorrected values are approximately equal after 7 samples are removed. Eq. 1.4, which was described by Bates et al (1966a), was used to calculate the corrected values.

$$C_n = C_{n,meas.} + \frac{5}{1500}\sum_{s=1}^{n-1}(C_{s,meas.}) \qquad \text{Eq. 1.4}$$

Where $C_{n,\ meas.}$ denotes the spectrophotometrically measured concentration (expressed as a % in this case), C_n is the concentration (% in this case) of the n^{th} sampling expected in the dialysis medium if the previous samples had not been removed and $\sum_{s=1}^{n-1}(C_{s,meas.})$ is the sum of concentrations (% in this case) measured spectrophotometrically from sample 1 to (n-1) sample. A corrected value (C_n) of 49.2% is obtained when Eq. 1.4 is applied to the data provided by formulation A after 7 samples have been removed. As can be seen from Table 1.4 the uncorrected value ($C_{n,meas.}$) is 48.6%, which is only 0.6% less than the corrected value and in the case of the aqueous solution (B) the difference is only 0.95%. In the case of the 1st,2nd,3rd,4th,5th and 6th samples the differences between corrected and uncorrected values will be less than 0.6% and 0.95% in the oily and aqueous systems, respectively.

(b) <u>From flask-stirrer method</u>

Tables 1.6 and 1.7 show the mean percentages of drug dissolved at different times in the flask-stirrer method when using nitro-

Table 1.6 Percent[a] nitrofurantoin dissolved at various times from different formulations using the flask-stirrer method.

Time (min) \ Formul[n]. (b)	A	B	C	D	E	F	G	H
5	20.0	21.8	79.8	80.3	11.9	5.6	12.6	11.0
10	40.5	38.7	84.2	86.2	20.5	10.4	25.9	19.3
20	68.1	51.5	86.9	88.4	31.5	16.0	46.0	29.0
30	77.1	65.4	88.6	90.1	38.5	26.5	57.2	40.0
45	82.7	74.8	88.6	90.3	49.3	39.4	66.5	46.8
60	84.2	84.1	88.9	90.1	57.2	51.2	73.9	55.9

Note
(a) Each value is the average of the results from 3 experiments.
(b) The formulations are 0.1% w/v nitrofurantoin in (A) FCO, (B) 20% w/v sucrose in FCO, (C) 0.25% w/v xanthan gum in distilled water, (D) 20% w/v sucrose + 0.25% w/v xanthan gum in distilled water, (E) 1% w/v Cab-o-sil in FCO, (F) 0.5% w/v aluminium stearate + 0.7% w/v lecithin + 0.35% w/v hydrogenated castor oil + 20% w/v sucrose in FCO, (G) 0.3% w/v Cab-o-sil + 20% w/v sucrose in FCO and (H) 1% w/v Cab-o-sil + 20% w/v sucrose in FCO.

Table 1.7 Percent[a] ampicillin dissolved at various times from different formulations using the flask-stirrer method.

Time (min) \ Formul[n]. (b)	A	B	C	D	E	F
5	67.6	35.5	80.6	81.3	13.2	15.1
10	80.5	47.6	99.9	99.4	20.3	28.6
20	97.3	63.4	101.2	100.9	29.6	43.0
30	99.0	69.5			35.3	55.2
45	98.1	82.3			46.3	69.8
60	98.6	92.1			55.3	79.9

Note
(a) Each value is the average of the results from 3 experiments.
(b) The formulations are 2% w/v ampicillin trihydrate in (A) FCO, (B) 30% w/v sucrose in FCO, (C) distilled water, (D) 30% w/v sucrose in distilled water, (E) 30% w/v sucrose + 1.25% w/v Cab-o-sil in FCO and (F) 0.5% w/v aluminium stearate + 0.35% w/v hydrogenated castor oil + 0.7% w/v lecithin + 30% w/v sucrose in FCO.

furantoin and ampicillin for each formulation, respectively. Plots of these percentages against sampling times, to give the dissolution rate curves for each formulation of nitrofurantoin and ampicillin, are given in Fig. 1.6 and 1.7, respectively. Only formulations D and F (Fig. 1.6) and C and E (Fig. 1.7) are shown and the rest of formulations lie between these two curves but have been omitted from the figures for the sake of clarity. However, their $^{t}50\%$ values are given in Tables 1.10 and 1.11 for nitrofurantoin and ampicillin, respectively. A cumulative correction was only made with ampicillin, using Eq. 1.4 mentioned in the previous method, since the corrected and uncorrected values are approximately equal in the case of nitrofurantoin.

The time required for 50% of the drug to appear in solution, i.e. $^{t}50\%$, calculated from individual dissolution rate curves for each formulation, was used as an index of the dissolution rate of nitrofurantoin and ampicillin. Analysis of variance and Duncan's test were carried out to distinguish the significance or otherwise of the differences between the mean $^{t}50\%$ values. Details of the analysis are described in Chapter 2 of the previous Section. The results are summarised in Table 1.8.

The results given in Table 1.8 show that the sensitivity of the flask-stirrer method in discriminating between the different formulations of both drugs was affected adversely by the low reproducibility of the results. In fact, the reproducibility appeared to be less satisfactory than with the dialysis method and probably resulted from the varying degrees of dispersion of different samples of a given formulation when it was added to the dissolution medium.

1.4 Discussion

(a) Dialysis method

The simplest test formulation that was used in this method was the

Fig 1.6 Plot of percent of nitrofurantoin dissolved versus time for an aqueous suspension containing 20% w/v sucrose plus 0.25% w/v xanthan gum (D) and an oily formulation corresponding to Stephens and Su's patent (1975) (F).

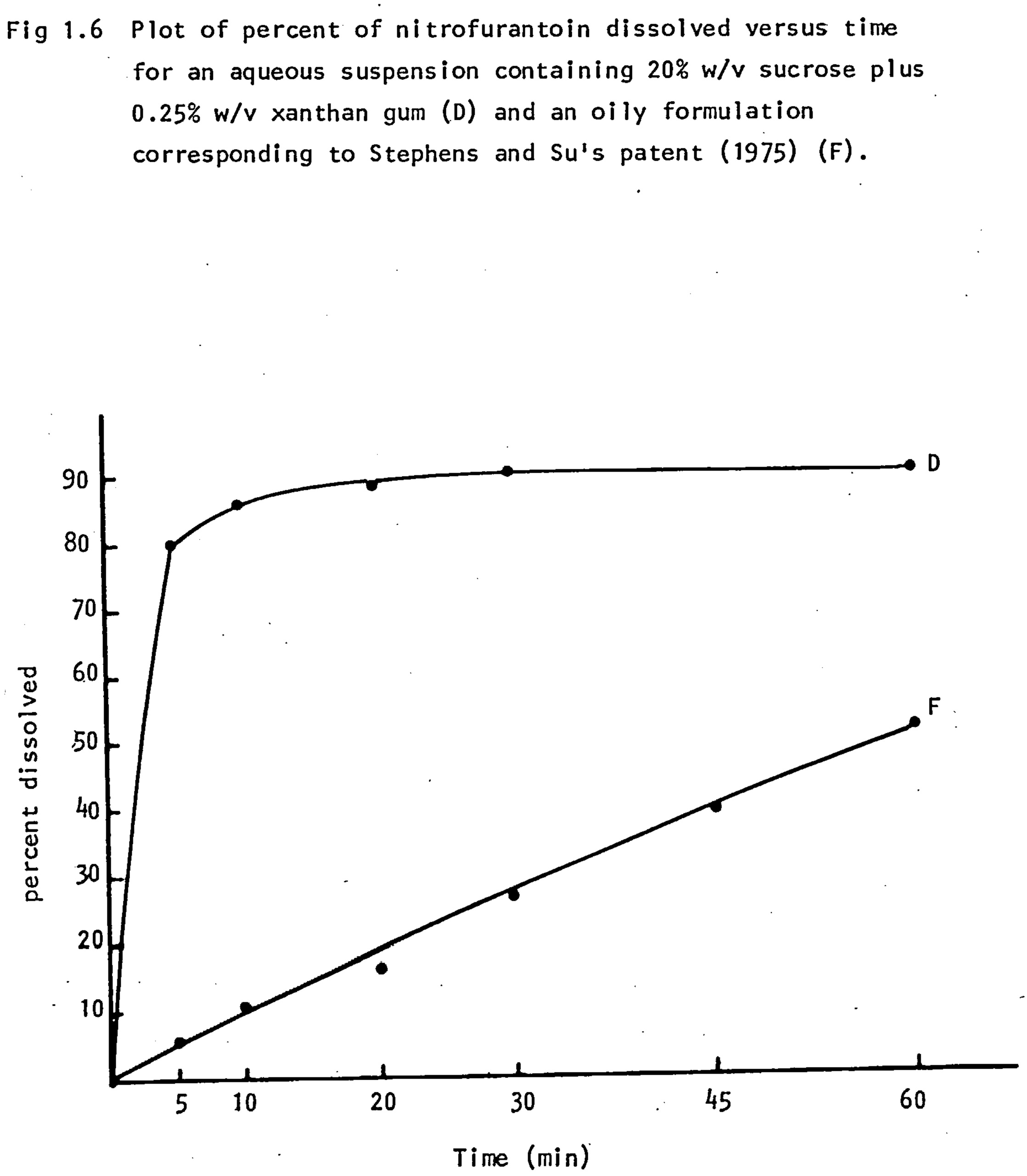


Fig 1.7 Plot of percent of ampicillin dissolved versus time for a simple aqueous suspension (C) and an oily suspension containing 30% w/v sucrose plus 1.25%w/v Cab-o-sil (E)

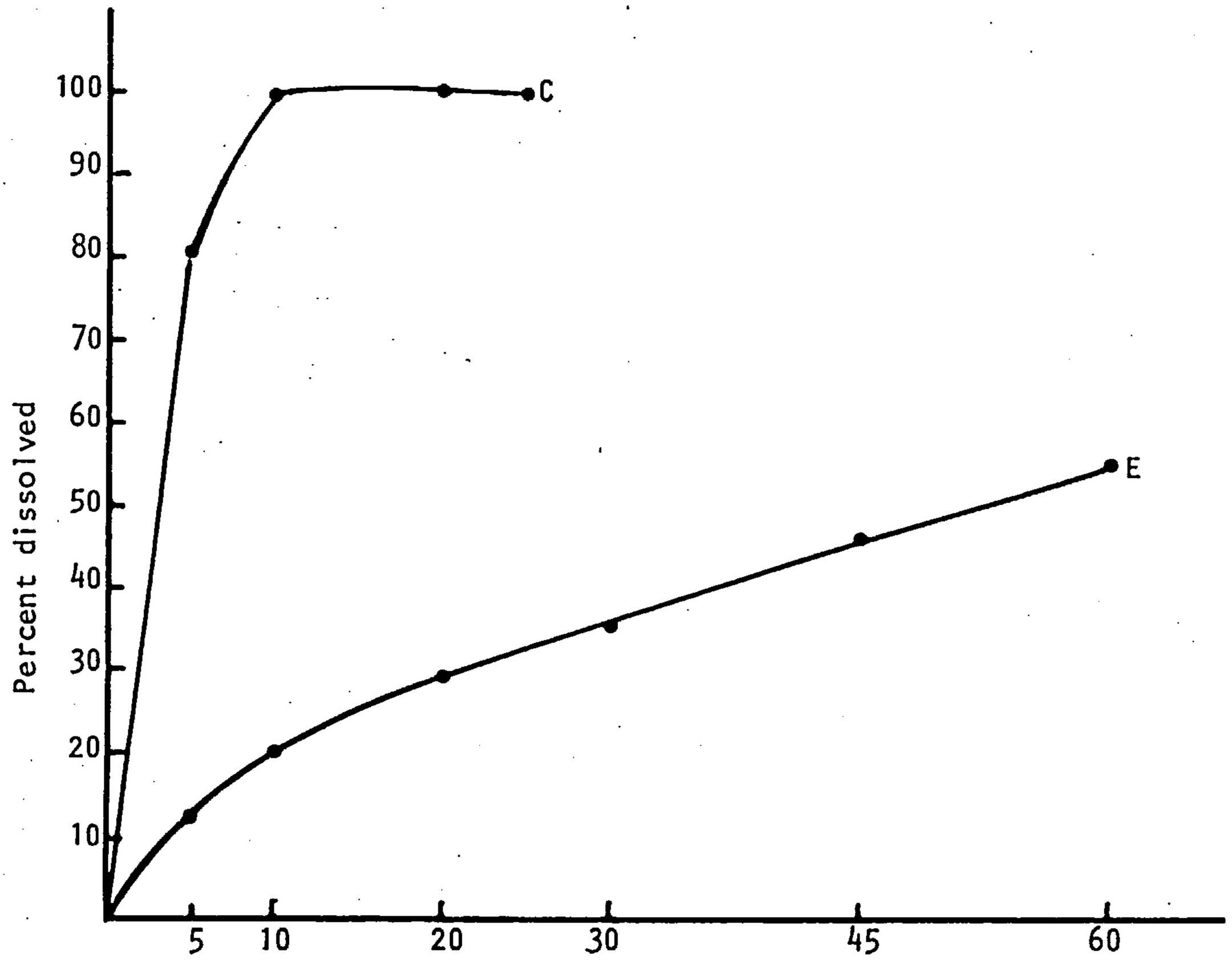


Table 1.8 Summary of the results of the statistical analysis of $t_{50\%}$ values for nitrofurantoin and ampicillin formulations.

(i) for nitrofurantoin

Mean values of $t_{50\%}$ in rank order	D	C	A	B	G	E	H	F
1% level	2.4	2.5	12.3	17.2	23.5	47.3	48.4	59.9
5% level	D	C	A	B	G	E	H	F

(ii) for ampicillin

Mean values of $t_{50\%}$ in rank order	C	D	A	B	F	E
1% level	3.00	3.00	3.6	12.3	25.7	51.0
5% level	C	D	A	B	F	E

where the underlinings have the same meaning as before.

(see Tables 1.6 and 1.7 for keys to nitrofurantoin and ampicillin suspensions, respectively.)

aqueous solution of sodium salicylate (formulation B). However, even in this system the overall release rate of the drug cannot be ascribed solely to the rate of dialysis of a single species in solution, because other reactions will be occurring as indicated by the simplified scheme in Fig. 1.8.

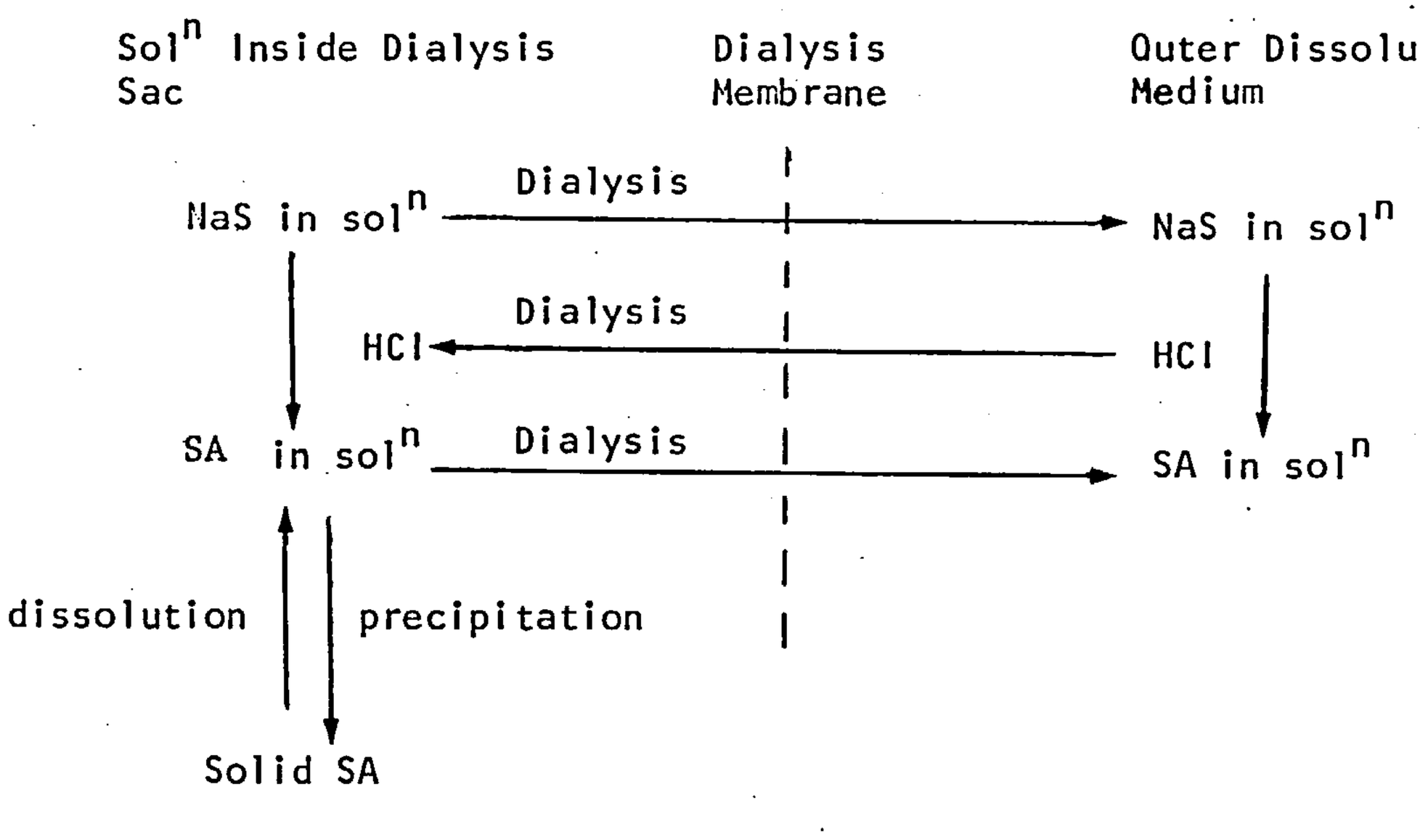


Fig. 1.8 Reactions occurring during dialysis of sodium salicylate solution into an acidic dissolution medium (NaS = sodium salicylate and SA = salicylic acid). NOTE. The effects of dissociation of the salicylic acid and its sodium salts are ignored in this scheme.

Thus, the observed rate of release will be influenced not only by the dialysis rates of salicylate ion and undissociated salicylic acid, but also by the rate of dissolution of any particles of precipitated salicylic acid. If this precipitation produces fine particles, then subsequent dissolution will be fairly rapid and the release rate will not be decreased markedly (Munzel, 1971). In fact, this aqueous solution provided the most rapid release rate of all the systems that were studied and gave mean $t_{30\%}$ and $t_{50\%}$ values

of 14.3 and 28.6 min, respectively (see Tables 1.5 and 1.9).

The mechanism of release of drug from the oily suspensions of sodium salicylate is likely to be more complex than from the aqueous solution, because (a) partition of salicylate between oily and aqueous phases must occur, (b) the oil can act as a reservoir for salicylic acid, formed by hydrolysis of the sodium salt and (c) some of the acidic aqueous dissolution medium penetrates through the dialysis membrane into the sac, particularly when sucrose is used as an ingredient in the oily vehicle. In addition, the release of water soluble compounds, such as sucrose into the aqueous dissolution medium, the sedimentation of sodium salicylate to the bottom of the oily vehicle inside the dialysis sac and the possibility of emulsification inside the dialysis sac may affect the release of salicylate.

In spite of the additional processes involved when oil alone is used as the suspension vehicle (formulation A) the initial rate of release of salicylate, as indicated by the $^{t}30\%$ value, is not much slower than from the aqueous solution B. The mean $^{t}30\%$ values are 22.2 min for A and 14.3 min for B but the statistical analysis of the results (see Table 1.5) indicated that this difference was insignificant at $p > 0.05$. It is possible that precipitation of salicylic acid from the aqueous solution B may be partly responsible for this similarity in the initial rates of release. However, the release rate from the oily suspension decreased at longer times in comparison with the aqueous solution and the $^{t}50\%$ values were $>$ 90 min for A and only 28.6 min for B. This difference may arise from the effect of the oil in formulation A acting as a reservoir for salicylic acid and so interfering with the apparent rate of appearance of salicylate in the aqueous dissolution medium. The difference in the later release of drug agrees with the results of Marty and Hersey (1975b), who found that the release rates

Table 1.9 Apparent viscosities, $t_{30\%}$ and $t_{50\%}$ values for sodium salicylate formulations.

Type of vehicle (a)	$t_{30\%}$ (min) (b)	η_{app} (mN s m^{-2}) (c)	$t_{50\%}$ (min) (b)
A	22.2	17.5	> 90
B	14.3	0.695	28.6
1A	34.0	37	
1B	37.1	50	
1C	45.3	59	
1D	50.7	69	
1E	51.5	81	
1F	58.3	92	> 90
1G	89.2	104	
1H	99.7	144	
1I	110	176	
2A	22.7	23	
2B	22.9	40	
2C	42.2	120	76.5
2D	41.1	105	87.5
3A	36.7	51	75.0
3B	35.3	83	71.0
3C	38.6	98	76.5
3D	44.4	131	> 90

(a) Formulations are as specified on pages 188 and 189.
(b) Each value is the mean of 3 experiments.
(c) η_{app}, apparent viscosity, from Table 1.2, Section 2.

of aqueous solutions of drugs were more rapid than those of oily suspensions in a dialysis system. This difference also indicates that the dialysis rate is not the rate limiting step in the release process and the method appears, therefore, to satisfy the conditions given by Swarbrick (1970) and Shah and Sheth (1976) for the determination of drug release rates from dosage forms using dialysis techniques.

Table 1.9 shows that, with few exceptions, a rank order correlation exists within each type of formulation (i.e. Types 1,2 or 3) between $t_{30\%}$ and the apparent viscosity of the different vehicles. In fact the correlation coefficient (r) for the values given by all the formulations is 0.8127 ($P < 0.001$). This rank order suggests that although the gels are affected by the presence of water, as mentioned in Section 2, the viscosity of the systems still retards the release of drug, at least in the early stages. This finding is in agreement with that reported by Buckwalter and Dickison (1948 and 1958) who found that increase in the concentration of aluminium stearate in the oil delayed the absorption of penicillin from an i.m. depot injection. They suggested that this is because of the entrapment of the drug particles within the gels. Although this suggestion seems to be in agreement with the results obtained in the present in vitro release studies viscosity appeared to play an insignificant role in the in vivo absorption studies on the oily vehicles (see Chapter 2, Section 3). (See also Chapter 1, Section 5 for further details on the correlation between in vivo and in vitro results).

It should be pointed out that, in addition to the poor reproducibility of the results that lowers the sensitivity of this dialysis method in discriminating between the release behaviour of the different formulations the model may be criticised due to the problems

that arise when there is an osmotic imbalance between the sac contents and the external dialysis medium, particularly with the formulations where sucrose is involved. It is possible that it may be desirable to add some aqueous fluid (0.1 mole/dm³ HCl) to the oily sample inside the dialysis sac to allow partitioning of drug from the oily to the aqueous phase before dialysis, because it is likely that the partitioning process is relevant to drug release in vivo (Kakemi et al, 1972a and b). Unfortunately,addition of the aqueous phase may cause a further complication in the system, particularly if emulsification occurs on stirring (Marty and Hersey, 1975a and b).

However, in the case of sucrose containing formulations (2C and D and 3A,B,C and D) osmotic imbalance between the inside and outside the dialysis sac caused the influx of appreciable volumes of the aqueous phase. In the earlier stages these formulations exhibited slow release rates compared with A and some other oily vehicles, (Table 1.9) probably because of their high viscosities. However, with the exception of formulation 3D the behaviour of which might be due to its very high viscosity and the adsorptive capacity of its Cab-o-sil content, these sucrose containing formulations were the only oily suspensions that gave $t_{50\%}$ values of $<$ 90 min. (see Table 1.9). These increases in release rate in the later stages were accompanied by the influx of aqueous phase, the volume of which was about the same as that of the oily liquid, i.e. 5 cm³, after 90 min. In the presence of water these oily vehicles form globules of different sizes (see part b of this discussion). Thus, the influx of water will not only affect the viscosity of the oily vehicle but will also enhance the rate of partition of drug between oil and water because of the increase in interfacial area between these two phases.

The results obtained from these in vitro studies suggest that the release of salicylate from a suspension of its sodium salt in FCO can be influenced by the inclusion of:

(a) aluminium stearate, which retards the release, particularly when its concentration is 1.5% w/v or more,

(b) sucrose, which tends to give rise to a faster release in the later stages, and

(c) Cab-o-sil, which tends to nullify the effects of sucrose, when used in a concentration of 1% w/v.

Other conclusions that can be drawn from these results include the fact that the inclusion of 0.7% w/v lecithin or 0.35 w/v hydrogenated castor oil in the oily vehicle does not appear to have any significant effects on the release process as shown by a comparison of the results obtained for formulations 2A and 2B, respectively, with that for the simple oily suspension (A). In fact these three oily formulations were the only ones that gave $t_{30\%}$ values that were not significantly different to that given by the aqueous solution of sodium salicylate (B). The $t_{50\%}$ values were, of course, all much greater than that of B. The insignificant effect of 0.7% w/v lecithin is also indicated by a comparison to the $t_{30\%}$ values obtained for formulations 2C and 2D.

Finally, there appears to be little difference in the in vitro release of salicylate from formulations that correspond to those patented by Stephens and Su (1975) and Lin and Pramoda (1978), as shown by a comparison of systems 2C and 3C, provided that the concentration of Cab-o-sil in Lin and Pramoda's formula does not exceed 0.5% w/v, because if 1.0% is used the increased rate of release, produced by sucrose in the later stages of release, is not so readily apparent.

(b) Flask-stirrer method

(i) Nitrofurantoin formulations

The results given in Table 1.8 show that the two suspensions in aqueous vehicles C and D produced rapid release of the drug and gave very close $t_{50\%}$ values. Both vehicles contained 0.25% w/v xanthan gum but D also contained 20% w/v sucrose. The presence of sucrose therefore appeared to have little effect on the release of nitrofurantoin from the aqueous suspensions. This is to be expected because the agitation of the dissolution medium will cause rapid dispersion and dilution of the dissolved sucrose.

The release of nitrofurantoin from the oily formulations was often poorly reproducible and this poor reproducibility was probably associated with the behaviours of the vehicles when they were placed in the dissolution medium. In addition, the variety of behaviours shown by the different vehicles was considered to be a major factor that contributed to the significant differences that were detected in the $t_{50\%}$ values from some of the vehicles.

In general the main difference in behaviours depended on the presence or absence of sucrose in the formulations. Thus, the formulations that did not contain sucrose, i.e. A (FCO only) and E (1% Cab-o-sil in FCO), tended to form oily layers on the surface of the dissolution medium. Under the influence of the agitation of the aqueous medium droplets of oil could be seen to become detached from the oily layer and then coalesce with it, particularly in the case of A. The higher viscosity of the layer formed by E reduced the tendency to form these droplets and was probably responsible for the significant increase in the $t_{50\%}$ value for this formulation over that of A, $p < 0.01$. The release of nitrofurantoin from the simple suspension in FCO (A) was fairly rapid as would be expected from the low oil: 0.1 mole/dm^3 HCl

partition coefficient of this drug (see next chapter). Because of the low reproducibility of the results, the mean $t_{50\%}$ value for this simple oily suspension did not differ statistically from the mean values for the aqueous suspensions C and D, $p > 0.05$.

In the cases of sucrose containing formulations, i.e. B (20% sucrose in FCO), G, (0.3% Cab-o-sil + 20% sucrose in FCO), H (1.0% Cab-o-sil + 20% sucrose in FCO) and F, which corresponded to Stephens and Su's patent (1975), the oily vehicle tended to form relatively large pear-shaped globules, in which the sucrose and other solid ingredients sedimented inside the globules leaving a clear oily layer at the top of the globule. The sizes of these globules ranged from approximately 1-10 mm and their overall densities caused them to fall to the bottom of the dissolution flask (see Fig. 1.2). As the sucrose was removed from the globules by dissolution into the aqueous phase they gradually disappeared and the oil then formed a layer on the surface of the aqueous phase. The lifetimes of these globules therefore appeared to depend on the dissolution rate of sucrose, which in turn will depend on the viscosity of the oily liquid inside the globules. Thus, the lifetime of the globules formed by B (20% sucrose in FCO) was only of the order of 5 minutes so that an oily layer was formed on the surface of the dissolution medium in a relatively short time. The viscosity of this layer was presumably similar to that of FCO alone and the release of nitrofurantoin from formulation B would therefore be expected to be not too much slower than from a suspension in FCO alone (A). In fact, the $t_{50\%}$ value for B was about 5 min longer than that for A and the two values were not statistically different, $p > 0.05$ (see Table 1.8).

The lifetimes of the globules produced by the remaining sucrose containing formulations fell into the order $G < H < F$. G and H also

contain Cab-o-sil in 0.3% and 1% concentrations, respectively. The resultant higher viscosities will delay the loss of sucrose from the globules and the release of nitrofurantoin not only from the globules but also from the oily layer that is eventually produced on the surface of the dissolution medium. In fact, the globule forming tendencies of these two formulations caused by their sucrose contents do not seem to be as important as the effect produced by including 1% Cab-o-sil, because formulations E and H gave very similar $t_{50\%}$ values (see Tables 1.8 and 1.10).

Table 1.10 Apparent viscosities and $t_{50\%}$ values for oily nitrofurantoin formulations

Formulations (a)	$t_{50\%}$ (b) (min)	η_{app} (c) mN s m^{-2}
A	12.3	17.5
B	17.2	51
E	47.3	58
G	23.5	83
F	59.9	120
H	48.4	131

Key:

(a) See Table 1.6 for key to formulations.
(b) Each value is the mean of 3 experiments.
(c) ηapp, apparent viscosity, from Table 1.2, Section 2.

These two formulations both contain 1% Cab-o-sil but only H contains 20% sucrose. Although the apparent viscosities of these two vehicles are markedly different (see Table 1.10), it is likely that the viscosities of the oily layers produced by both of them will be similar when the sucrose has been removed from H. The increase in viscosity of an oily layer produced by 0.3% Cab-o-sil does not appear

to be sufficient to lead to a significant decrease in the release rate of nitrofurantoin because the $t_{50\%}$ value of formulation G did not differ significantly from that of B, $p > 0.05$.

Finally, although the apparent viscosity of formulation F, which corresponds to the patent of Stephens and Su (1975), was less than that of H (see Table 1.10) the lifetime of the globules was greater in F. This increase in globule's lifetime appears to be responsible for the higher mean $t_{50\%}$ value for F compared to H, and the difference between these values was significant, $p < 0.05$ (see Table 1.8).

One final point concerning the aqueous suspensions of nitrofurantoin (C and D) is that the maximum amount of drug released appeared to be about 90% and this value remained constant over the last few sampling times. This may suggest that some nitrofurantoin remains in the oily vehicle but the low oil: 0.1 mole/dm^3 HCl partition coefficient does not support this suggestion. The decomposition of nitrofurantoin under the influence of light offers an alternative explanation. Such decomposition was confirmed by preliminary studies and although the dissolution experiments were carried out in the dark as far as possible, exposure to light could not be avoided completely. Thus, some decomposition of dissolved nitrofurantoin is likely to occur and may reduce the rate of apparent dissolution of the drug particularly in the latter stages when the concentration of nitrofurantoin in solution is high and when the rate of dissolution is decreasing.

(ii) <u>Ampicillin formulations</u>

The results obtained with these formulations (Table 1.7 and 1.8) parallel those obtained with the nitrofurantoin ones. This is not surprising because both drugs possess low oil: 0.1 mole/dm^3 HCl partition coefficients (see next chapter). Thus, the aqueous suspensions

(C and D) gave the lowest $t_{50\%}$ values and the inclusion of 30% sucrose in D did not cause any apparent effect. The $t_{50\%}$ value for the suspension in FCO alone could not be distinguished statistically from those of the aqueous formulations, $p > 0.05$, and neither could that for the oily suspension B, which contained 30% sucrose. The low lipophilicity of ampicillin trihydrate was visually apparent in these systems because the solid particles could be seen to be released from the FCO in formulation A and fall through the dissolution medium in the same way as they did with the aqueous suspensions. It is, therefore, not surprising that the $t_{50\%}$ value for this formulation should be very similar to those of the aqueous suspensions C and D.

The formulation F, which corresponds to Stephens and Su's patent (1975) gave a significantly longer $t_{50\%}$ value than the simple oily suspension A, $p < 0.01$, but was not statistically distinguishable at $p > 0.01$ from formulation B, which contained sucrose. This latter difference was significant however at the 5% level. It should be pointed out that the sucrose content of the appropriate ampicillin formulations was 30% rather than 20% as in the previous nitrofurantoin suspensions. Finally, the longest $t_{50\%}$ value was given by formulation E, which contained 30% sucrose plus 1.25% Cab-o-sil, so that not only was the sucrose content higher than that in the nitrofurantoin suspensions but also the Cab-o-sil content was increased. This formulation had a very high apparent viscosity, as shown in Table 1.11. The values given in this table also show that there is an approximate rank order relationship between $t_{50\%}$ and $\eta_{app.}$

Like the nitrofurantoin formulations, the ampicillin suspensions B, F and E formed large pear-shaped globules and the lifetimes of these appeared to be a major factor in determining the release of ampicillin.

Table 1.11 Apparent viscosities and $t_{50\%}$ values for oily ampicillin formulations

Formulation (a)	$t_{50\%}$ (b) (min)	η_{app} (c) (mN s m^{-2})
A	3.6	17.5
B	12.3	64
F	25.7	140
E	51.0	150

Key:

(a) See Table 1.7 for key to formulations.
(b) Each value is the mean of 3 experiments.
(c) η_{app}, apparent viscosity, from Table 1.2, Section 2.

In addition, the dispersion of the vehicles into these globules and the formation of oily layers on the surface of the dissolution medium in an uncontrollable manner was probably responsible for the poor reproducibility of the results. Although this poor reproducibility may be a criticism of the method used to determine the release of drug it is likely that a similar phenomenon will occur <u>in vivo</u> in the gastric fluids.

CHAPTER 2

SOLUBILITY, PARTITION COEFFICIENT AND ADSORPTION STUDIES

2.1 Introduction

Since the absorption of drugs occurs normally from solution (Morrison and Campbell, 1965; Cadwallader, 1974), aqueous solubility and, consequently, the amount of drug in solution are of importance with respect to drug activity. However, lipid solubility is equally important since the absorption involves the passage of the drugs through biological membranes, which are lipoidal in nature (see part 1.3.1, Section 1). A guide to the lipophilic nature of a drug is provided by its partition coefficient between oil or a fat-like solvent, such as chloroform, and aqueous phase, which is either water, dilute HCl or an aqueous buffer approximating the pH of the absorption site. The effect of lipid solubility on absorption was studied by Schanker (1960) for a series of barbituric acid derivatives. Each compound had about the same pK_a value and an almost perfect rank order correlation was found to exist between their partition coefficients and extents of absorption.

Physical adsorption involves the removal of drug molecules from solution and their transfer to the surface of an "active" solid, such as charcoal, alumina, various clays or colloidal silicon dioxide. The molecules are held on the surface by van der Waal's forces or hydrogen bonding. An equilibrium between drug in solution and adsorbed drug is usually observed. When adsorption is irreversible, a chemical adsorption (chemisorption) rather than a physical adsorption is suggested.

A number of pharmaceutical adjuvants and antidiarrhoeal medications may function as "active" solids or adsorbents. The ability of certain adsorbents to interfere with drug absorption is well known.

The use of charcoal as a non-specific antidote in drug poisoning is based on its adsorption properties and its consequent ability to remove a portion of the drug from the GI fluids. The poor serum levels of lincomycin obtained upon co-administration with attapulgite-pectin suspension have been attributed to an adsorption process (Monkhouse and Lach, 1972). Sorby (1965) showed that human urinary levels of promazine are significantly decreased when the drug is adsorbed on to attapulgite and charcoal prior to administration.

With the above points in mind the purpose of this work was to study the solubility of the drugs in both 0.1 mole/dm^3 HCl and the oil, their partition coefficient between the oil and HCl and their adsorption from solution on to Cab-o-sil.

2.2 Experimental

2.2.1 Materials

Details of the sources of materials and the methods of preparation of Cab-o-sil dispersion are given in the previous sections of this thesis.

2.2.2 Methods

(a) Solubility studies in 0.1 mole/dm^3 and FCO

An excess amount of sodium salicylate or nitrofurantoin were added to 100 cm^3 of the particular solvent and kept at 37^{o}C for a week with occasional shaking. Samples were taken periodically from the supernatant solution to predict the equilibrium solubility, filtered through a 0.45 Millipore filter and then diluted to an appropriate extent with the particular solvent. The concentration of the drug (solubility) was calculated from the previously made calibration curves in the appropriate solvent. Calibration curves in the HCl are given in the previous chapter. Tables 2.1 and 2.2 show the concentrations of standard solutions of sodium salicylate and nitrofurantoin in FCO

and their absorbance values at 298 nm and 363 nm, respectively, that were used to construct the necessary calibration curves. The concentrations of the test solutions were calculated by means of Eq. 2.2 and 2.3. Equilibrium solubility was attained in 3 days in all cases. The experiment was carried out in the dark with nitrofurantoin.

Table 2.1 Data for calibration curve of sodium salicylate in FCO at 298 nm.

Concentration (X) mg/100 cm³	Absorbance Y
0.2	0.051
0.4	0.110
0.6	0.162
0.8	0.211
1	0.275

$$X = \frac{(Y - \bar{Y}) + b\bar{X}}{b} \qquad \text{Eq. 2.1}$$

$$X = \frac{Y + 0.0039}{0.2795} \qquad \text{Eq. 2.2}$$

Table 2.2 Data for calibration curve of nitrofurantoin in FCO at 363 nm.

Concentration (X) mg/100 cm³	Absorbance (Y)
0.2	0.182
0.4	0.381
0.6	0.582
0.8	0.766
1.00	0.962

$$X = \frac{Y + 0.0089}{0.9725} \qquad \text{Eq. 2.3}$$

(b) Oil/0.1 mole dm^{-3} HCl partition coefficient

50 cm^3 of solutions containing 100 mg of sodium salicylate or ampicillin or 1 mg of nitrofurantoin in 100 cm^3 of 0.1 mole/dm^3 HCl were equilibrated with 50 cm^3 of FCO for 24 hr in a 250 cm^3 glass stoppered conical flask kept at 37°C in a shaking water bath and agitated at 100 oscillations per min. The experiment was carried out in the dark with nitrofurantoin. The drug concentrations in the HCl were determined according to the appropriate method given in the previous chapter. Preliminary studies showed that equilibrium was attained within 5 hr in all cases. The apparent partition coefficient of each drug was calculated by means of the following equation:

$$\text{App. partition coefficient} = \frac{C_1 - C_2}{C_2} \qquad \text{Eq. 2.4}$$

where C_1 is the original concentration of the drug in the HCl and C_2 is the equilibrium concentration in the HCl.

(c) Adsorption studies

0.5 g quantities of Cab-o-sil were placed in 100 cm^3 glass stoppered conical flasks containing 50 cm^3 of sodium salicylate or nitrofurantoin solutions of specified concentrations in FCO (see Tables 2.4 and 2.5 respectively). The flasks were shaken in a 37°C shaking water bath at 100 oscillations per min for 24 hr. Preliminary experiments had shown that equilibrium was attained in less than 5 hr. The drug concentrations in the supernatant solutions were determined after centrifugation at 8000 r.p.m. for 10 min using Eqs. 2.2 and 2.3 mentioned above. The experiment was carried out in the dark with nitrofurantoin.

2.3 Results

Table 2.3 shows the solubilities of the drugs in the 0.1 mole/dm³ HCl and FCO together with their oil/HCl partition coefficients. Corrections of the absorbances of the aqueous solutions of the drugs to account for the effect of water soluble components of the oil were not made, because preliminary experiments showed that these effects were negligible.

The adsorption data for sodium salicylate and nitrofurantoin on to the Cab-o-sil are shown in Tables 2.4 and 2.5, respectively.

Table 2.3 Solubilities and partition coefficients of sodium salicylate, nitrofurantoin and ampicillin. Each value is the average of 3 experiments.

	Solubility (mg/100 cm³)		apparent
	0.1 mole/dm³ HCl	FCO	partition coefficient
Sodium salicylate	306.1	16.85	38.60
nitrofurantoin	15.59	4.47	0.48
ampicillin	>2000 (a)	< 3	0.052

(a) Marsh and Weiss (1967)

Table 2.4 Adsorption data for sodium salicylate on to Cab-o-sil from solution in FCO. Each value is the average of 2 experiments.

c_o mg/100 cm³	c_{eq} mg/100 cm³	x/m mg/g
0.5	0.47	0.03
1	0.89	0.11
1.5	1.27	0.23
2	1.55	0.45
3	2.19	0.81
4	2.76	1.24
5	3.40	1.60
7	4.41	2.59
10	6.04	3.96
15	8.90	6.10

c_o is the original concentration of drug in solution
c_{eq} is the equilibrium concentration of drug in solution
x is the amount of drug adsorbed by mass m of the adsorbent.

Table 2.5 Adsorption data for nitrofurantoin on to Cab-o-sil from solution in FCO. Each value is the average of 2 experiments.

c_o mg/100 cm³	c_{eq} mg/100 cm³	x/m mg/g
0.2	0.20	0.0
0.4	0.395	0.005
0.6	0.590	0.01
0.8	0.78	0.02
1	0.97	0.03
1.5	1.46	0.04
2.00	1.97	0.03
4.00	3.95	0.05

c_o, c_{eq}, x and m have the same meaning as in Table 2.4

2.4 Discussion

Contrary to the partition coefficient expected on the basis of the solubilities of sodium salicylate in FCO and 0.1 mole/dm³ HCl, the relatively high apparent oil/HCl partition coefficient (Table 2.3) indicates the effect of conversion of this drug to the very lipophilic salicylic acid in the acidic medium. This effect will also occur in the stomach and would be a possible explanation, besides the delay in the GER caused by the oil, for the slower absorption rate *in vivo* (see Chapter 1, Section 3) and for the slower release rate *in vitro* (previous chapter).

The solubilities and partition coefficients of nitrofurantoin and ampicillin are shown in Table 2.3. A solubility of 15.59 mg/100 cm³ of nitrofurantoin in 0.1 mole/dm³ HCl is very close to that found by Bates et al (1974a) (15.4 mg/100 cm³). However, its solubility in FCO (4.47 mg/100 cm³) is nearly double that found by the same authors (2.07 mg/100 cm³) for its solubility in peanut oil. The apparent oil/HCl partition coefficient of nitrofurantoin is less than unity, 0.48.

Ampicillin is almost insoluble in all organic solvents (Marsh and Weiss, 1967). Its ethylacetate-water partition coefficient is 0.044 (Hou and Poole, 1969). Its solubility in FCO was found to be <3 mg/100 cm³ and the apparent oil/HCl partition coefficient is 0.052, Table 2.3.

The adsorption studies indicate that sodium salicylate is significantly adsorbed on to Cab-o-sil from solution in FCO (Table 2.4) but no appreciable adsorption of nitrofurantoin occurs on to this suspending agent (Table 2.5).

It has been reported that the use of colloidal silicon dioxide as a viscosity modifier is largely attributed to the ability of the very small silica particles to form a network structure throughout the medium by interparticle hydrogen bonding via the silanol groups on the silica

surface. In addition to these particle interactions there is possible bonding between the silanol groups and other components that are also capable of hydrogen bond formation (Marshall and Rochester, 1975). The amount of drug adsorbed per gram of Cab-o-sil will consequently depend on the strength of any interactions, such as hydrogen bond formation, between the drug and the surface silanol groups relative to the competing effects of silanol groups on other particles or of compounds, such as fatty acids, that are present in FCO. The relative numbers of these competing agents will also be important. For example, Sherriff and Enever (1979) showed that methyl salicylate could bond to the silanol groups of Cab-o-sil when n-dodecane was used as the dispersion medium but not when l-dodecanol was used, because in the latter systems there was such an excess of hydroxyl groups from the l-dodecanol relative to the silanol groups that the probability of silanol-l-dodecanol interactions was even higher than that of silanol-silanol interaction. Thus, there was no chance for methyl salicylate to be adsorbed.

Increase in the number of surface silanol groups, i.e. by using more Cab-o-sil, will obviously lead to relatively greater adsorption of a fixed amount of drug. This may provide a likely explanation of the fact that 1% Cab-o-sil nullified the enhancing effect of sucrose on the bioavailability of sodium salicylate whilst 0.3% Cab-o-sil allowed the effect to be retained (see Chapter 2, Section 3).

The adsorption of salicylate by Cab-o-sil may also be responsible for some of the effects observed in the in vitro studies as indicated in the previous chapter.

The negligible adsorption of nitrofurantoin by Cab-o-sil (Table 2.5) indicates that the effects of this suspending agent in the in vivo and in vitro studies were unlikely to involve any adsorption phenomena.

SECTION 5

IN VIVO - IN VITRO CORRELATIONS AND CONCLUDING REMARKS

CHAPTER 1

CORRELATION OF IN VIVO AND IN VITRO RESULTS

It is suggested, as indicated in Section 3, that the differences obtained in the bioavailabilities of the three drugs studied were mainly due to the decrease in the GER brought about by the oil or by the high osmotic pressure produced by sucrose, although other possible physiological factors mentioned in that section might also be involved. The effects of all the physiological functions, particularly GER, masked the inherent effect of the suspending agents and the viscosity of the oily vehicles for the range of concentrations studied. Therefore, it is not surprising that the lack of factors similar to these physiological effects in the *in vitro* dissolution and release studies resulted in poor correlation between the *in vivo* and *in vitro* parameters (see part 1.2.3, Section 1). However, there are instances where some correlations were observed,as will be seen later in this chapter.

1.1 Sodium salicylate

It was suggested in Chapter 2, Section 3 that the results obtained *in vivo* cannot be correlated with the viscosity of the formulations and that the effect of the oil on the GER predominates. In fact, analysis of the results in Table 1.1 shows that of the three *in vivo* parameters, i.e. AUC_o^9, PC and PT, only PT gives any reasonable degree of correlation with viscosity as indicated by Eq. 1.1 - 1.3 respectively.

$$AUC_o^9 = 100.92 - 0.0722\ \eta_{app} \qquad \text{Eq. 1.1}$$
$$r = -0.426,\ p > 0.1$$

$$PC = 13.938 - 0.0107\ \eta_{app} \qquad \text{Eq. 1.2}$$
$$r = -0.4454,\ p > 0.1$$

$$PT = 2.493 + 0.0164\ \eta_{app} \qquad \text{Eq. 1.3}$$
$$r = 0.7409,\ p < 0.05$$

Table 1.1 In vivo and in vitro parameters for sodium salicylate formulations together with the viscosities of the various vehicles.

Formulation (a)	η_{app} (b) (mN S m^{-2})	AUC_o^9 (mg hr/100 cm^3)	PC (mg/100 cm^3)	PT (hr)	$t_{30\%}$ (min)
A	17.5	89.8	12.7	2.7	22.2
C	50	96.9	12.8	3.2	37.1
B	51	106	14.7	2.7	36.1
D	83	102	13.9	4.6	38.6
G	105	92	12.7	5.5	41.1
F	120	99	13.8	4.0	42.2
E	131	86	12.0	4.25	44.4
H	144	85	11.4	4.5	99.7

(a) Formulations are as specified in Table 2.1, Section 3.
(b) η_{app}, apparent viscosity, from Table 1.2, Section 2.

The latter correlation (i.e. PT with viscosity) probably arises from the fact that salicylate is absorbed fairly well from the stomach (see Chapter 1, Section 3) and that an increase in the viscosity would decrease the rate of movement of drug molecules to the absorbing membrane (Levy and Jusko, 1965). The effect of viscosity on the in vitro release rate of salicylate is shown by the existence of a correlation between viscosity and the $t_{30\%}$ as shown by Eq. 1.4:

$$t_{30\%} = 13.160 + 0.3651\ \eta_{app} \qquad \text{Eq. 1.4}$$
$$r = 0.7113,\ p < 0.05$$

Analysis of the results also showed that the three in vivo parameters did not correlate with the in vitro parameter $t_{30\%}$ ($p > 0.1$). However, in spite of this lack of correlation formulation E (i.e. 1% w/v Cab-o-sil + 20% w/v sucrose in FCO) which gave the lowest but one AUC_o^9 value, also gave the highest but one $t_{30\%}$ value, i.e. almost the slowest release rate. Two possible explanations might be suggested to account for

this correlation, i.e. the high viscosity of this formulation (Table 1.1) and the possible adsorption of salicylate on to the Cab-o-sil. However, because of the poor correlation between viscosity and AUC_o^9 (Eq. 1.1) and the marked adsorption of this drug on to Cab-o-sil, as indicated by the adsorption studies (see Chapter 2, Section 4), it is suggested that the decreased in vivo and in vitro availabilities of sodium salicylate when administered in this formulation are most likely due to the adsorption of the drug on to Cab-o-sil.

Finally, the rapid absorption of salicylate from the aqueous solution compared with that from the oily suspension (Chapter 1, Section 3) correlates well with the rapid in vitro release rate of this drug from the former formulation (Chapter 1, Section 4). This latter correlation reflects the action of oil as a reservoir for a drug with high oil/0.1 mole/dm³ HCl partition coefficient, e.g. salicylic acid.

1.2 Nitrofurantoin

It was suggested in Chapter 3 of Section 3 that the significant reduction in the rate and extent of nitrofurantoin absorption when administered in oily rather than aqueous suspensions is due to the effect of the oil on the GER and that the viscosity of the vehicle played an insignificant role. In fact, analysis of the results given in Table 1.2 supports this suggestion by showing poor correlations between viscosity and either the amount of drug excreted (as % dose) during the first 4 hr and 8 hr periods or the total amount excreted, as indicated by Eq. 1.5 - 1.7, respectively.

$$\%\ \text{dose excreted (4 hr)} = 17.746 - 0.1006\ \eta_{app} \qquad \text{Eq. 1.5}$$
$$r = -0.5907,\ p > 0.1$$

$$\%\ \text{dose excreted (8 hr)} = 23.238 - 0.1009\ \eta_{app} \qquad \text{Eq. 1.6}$$
$$r = -0.5453,\ p > 0.1$$

$$\% \text{ dose (Total)} = 27.62 - 0.0642\ \eta_{app} \qquad \text{Eq. 1.7}$$
$$r = -0.5105,\ p > 0.1$$

However, viscosity appears to play a significant role in the <u>in vitro</u> dissolution studies, as indicated by the existence of a correlation between $t_{50\%}$ and viscosity (Eq. 1.8)

$$t_{50\%} = -2.7560 + 0.444\ \eta_{app} \qquad \text{Eq. 1.8}$$
$$r = 0.8244,\ p < 0.02$$

Table 1.2 In vivo and in vitro parameters for nitrofurantoin formulations together with the viscosities of the various vehicles.

Formulation (a)	η_{app} (mN s m^{-2})	Nitrofurantoin excreted (% dose) 4hr	8hr	Total	$t_{50\%}$ (min)
A	17.5	7.8	10.7	20.2	12.3
C	33	21.7	30.1	31.7	2.4
D	37	22.8	28.2	31.5	2.5
B	51	9.1	13.1	22.6	17.2
E	58	7.8	13.5	20.2	47.3
G	83	8.9	13.2	21.1	23.5
F	120	5.4	8.3	18.6	59.9
H	131	5.1	10.5	21.0	48.4

(a) Formulations are as specified on page 146.

In addition, $t_{50\%}$ showed reasonable degrees of correlation with the <u>in vivo</u> parameters as indicated by Eq. 1-9 - 1.11.

$$\% \text{ dose excreted (4hr)} = 17.627 - 0.2455\ t_{50\%} \qquad \text{Eq. 1.9}$$
$$r = -0.776, p < 0.05$$

$$\% \text{ dose excreted (8hr)} = 23.244 - 0.2733\ t_{50\%} \qquad \text{Eq. 1.10}$$
$$r = -0.7302, p < 0.05$$

$$\% \text{ dose excreted (Total)} = 28.071 - 0.1764\ t_{50\%} \qquad \text{Eq. 1.11}$$
$$r = -0.7556, p < 0.05$$

These correlations are perhaps surprising in view of the previous comments that the <u>in vivo</u> parameters do not show any relationship to viscosity whereas the <u>in vitro</u> parameter $t_{50\%}$ does show some correlation

with viscosity. The only explanation that can be suggested to account for this apparent contradiction is that dissolution, whether in vivo or in vitro, is influenced not only by the viscosity of the formulation but by additional factors, e.g. possible effects of the formulation components on the solubility of the nitrofurantoin, adsorption of nitrofurantoin on to sucrose particles in the oily formulations or complexation with sucrose in solution in the aqueous phase. The correlation between the in vitro dissolution rate and the in vivo parameters is supported by the work of Groning (1981) who studied the bioavailability of nitrofurantoin after oral administration of dosage forms with different onsets of release. The author found that a delay in the release of merely a few hours leads to a statistically significant reduction in the bioavailability of active ingredients. After administration of coated tablets, where release was delayed for up to 5 hr, only 8.3% of the dose was excreted in the urine, whereas with rapidly disintegrating tablets, 34.5% of the dose underwent renal elimination. The author indicated that nitrofurantoin is only optimally available from the GI tract over a limited period and that with dosage forms of nitrofurantoin, which are subject to passage through the GI tract, only that part of the active ingredient which is released from the preparation within the first few hours of administration is optimally absorbed and eliminated in the urine.

1.3 Ampicillin

The AUC_0^8 and PT values correlate poorly with viscosity as indicated by Table 1.3 and Eq. 1.12 and 1.13. However, the existence of a reasonable correlation between PC and viscosity (Eq. 1.14) is probably due, as for salicylate, to the gastric absorption of ampicillin, so that the higher the viscosity the slower absorption of the drug (Levy and Jusko, 1965). The effect of viscosity on the in vitro dissolution and

Table 1.3 In vivo and in vitro parameters for ampicillin together with the viscosities of the various vehicles

Formulation (a)	η_{app} (mN s m^{-2})	AUC µg hr/cm^3	PT (hr)	PC µg/cm^3	$t_{50\%}$ (min)
C	0.695	12.0	1.0	5.2	3.0
D	2.32	18.6	1.2	6.4	3.0
A	17.5	20.1	2.1	4.9	3.6
B	64	17.4	1.3	3.8	12.3
F	140	15.3	1.8	3.7	25.7
E	150	13.2	1.9	3.5	51.0

(a) Formulations are as specified in Table 4.1, Section 3.

release of ampicillin is shown by the existence of correlation between $t_{50\%}$ and viscosity (Eq. 1.15).

$$AUC_o^8 = 17.155 - 0.0169\ \eta_{app} \qquad \text{Eq. 1.11}$$

$$r = -0.3642,\ p > 0.1$$

$$PT = 1.334 + 0.003456\ \eta_{app} \qquad \text{Eq. 1.13}$$

$$r = 0.5322,\ p > 0.1$$

$$PC = 5.463 - 0.0141\ \eta_{app} \qquad \text{Eq. 1.14}$$

$$r = -0.851,\ p < 0.05$$

$$t_{50\%} = 0.373 + 0.2573\ \eta_{app} \qquad \text{Eq. 1.15}$$

$$r = 0.917,\ p < 0.01$$

Analysis of the results also showed that the three <u>in vivo</u> parameters did not correlate with the <u>in vitro</u> parameter $t_{50\%}$ ($p > 0.1$ for AUC_o^8 and PT and $p > 0.05$ for PC).

Although there is a poor correlation between $t_{50\%}$ and the <u>in vivo</u> parameters, the slower release rates of ampicillin from the formulations E and F (i.e., 1.25% Cab-o-sil + 30% sucrose in FCO and the formulation patented by Stephens and Su, (1975), respectively) are paralleled by the lower bioavailabilities of this drug from these 2 formulations <u>in vivo</u> (see Chapters 4 and 1 in Sections 3 and 4, respectively).

In conclusion, these *in vivo* - *in vitro* correlation studies indicate and confirm the suggestion made in Section 3 that the viscosity enhancing agents have no significant effect on the bioavailability of the 3 drugs studied when administered in an oily vehicle, since the major effect of the oil in decreasing GER masks the other effects of viscosity. However, viscosity did affect, and correlated with, the *in vitro* dissolution and release rates of these drugs. Finally, it is suggested that the correlations found between PT or PC and viscosity in the cases of salicylate and ampicillin, respectively, are related to the appreciable gastric absorption of these 2 drugs, because their rates of absorption are likely to be affected by the rates at which they arrive at the gastric mucosa.

CHAPTER 2

CLINICAL SIGNIFICANCE OF THE RESULTS AND SUGGESTIONS FOR FURTHER WORK

The results of the present investigations suggest that the bioavailability of drugs in perorally administered suspensions is greatly affected by the physicochemical properties of the vehicle and the subsequent action of this vehicle on the physiological functions as well as the properties of the drug. For example, oils and osmotic pressure decrease the GER, which is very important in the case of acidic drugs, e.g. salicylate and nitrofurantoin, and for an amphoteric drug, such as ampicillin. In addition, the increased bile secretion caused by the presence of oil,is suggested to be responsible for enhancement of the enterohepatic recycling process for drugs that are excreted extensively in the bile in active form with minimum metabolism by the liver cells, such as ampicillin. Furthermore, the effects of oil and osmotic pressure on the bioavailability of the acidic drugs appeared to depend, in turn, on the degree of acidity of these drugs and their oil/HCl (0.1 mole/dm^3) partition coefficients. For example, in the case of sodium salicylate, which forms salicylic acid (pKa = 3) as a result of the acidic medium of the stomach, a decrease in GER caused an increase in the extent of drug absorbed and the blood level remained rather high for a longer period of time when administered in oily suspension rather than aqueous solution. However, the reduction in the rate of absorption is due partly to the action of the oil as a reservoir that controls the release of salicylic acid and partly to the delay in the appearance of salicylate in the small intestine because of the decrease in the GER.

Another important conclusion is that the inclusion of sucrose in the oily vehicle increased the extent of absorption of drugs with oil/HCl (0.1 mole/dm^3) partition coefficients of more than unity due to its osmotic pressure effect on the uptake of water by the GI membrane and

not to any additional decrease in GER over that caused by the oil itself. Evidence for this conclusion arises from the fact that this was the case with sodium salicylate with an apparent oil/HCl partition coefficient of 38.6, whereas with ampicillin, with a partition coefficient of 0.052, there was no enhancement in the extent of absorption when sucrose was included in the oily vehicle, although 30% w/v was used instead of 20% w/v. However, inclusion of 1% w/v Cab-o-sil nullified the enhancement effect of sucrose on the extent of absorption of sodium salicylate. Adsorption of salicylate on to the large surface area of Cab-o-sil was found in *in vitro* studies and it is suggested that this adsorption decreased the availability of salicylate *in vivo* and the *in vitro* release rate.

The extent of absorption of ampicillin was enhanced by inclusion of sucrose in the aqueous vehicle (distilled water) to a value close to those given by the simple oily vehicle and the oil with sucrose. It is concluded that sucrose exerts its action on GER in aqueous systems but produces no additional decrease over that caused by the oil itself, unless there is a certain concentration beyond which an additional decrease in GER will be observed. It is obvious, therefore, that this point should be borne in mind for future work. It is recommended, therefore, that more attention should be paid to the osmotic pressure, since this effect might modify the absorption of drugs from their dosage forms.

However, with very weakly acidic and less water soluble drugs, e.g. nitrofurantoin (pK_a = 7.2), opposite results are apparently obtained, i.e. a reduction in the extent of absorption as a result of the decrease in GER together with a decrease in the rate of absorption as indicated by the percentage of the dose excreted in the urine. The reasons for these differences have already been discussed. The inclusion of sucrose in

oily vehicles or increasing their viscosities did not give any further change in the bioavailability of nitrofurantoin thus suggesting that the effect of the oil is predominant.

The studies presented in this thesis relate only to a small number of drugs and a small range of the viscosity of the oily vehicle. Extension of these studies to include other drugs with different solubilities and pK_a values would be required before the relation between the effects of oil or osmotic pressure and the properties of a drug, that are implied by the results obtained to date, could be substantiated. Since the effects of viscosity and osmotic pressure were masked by the predominant effect of the oil, it would be worthwhile studying a wider range of viscosities and higher concentrations of sucrose in the oil to detect any critical values beyond which these two effects might appear.

It is suggested that the effect of oil on the bioavailability of the drugs studied is mostly due to its action in decreasing GER. However, studies on the other actions of the oil (see Chapter 1, Section 3) and the significance of these actions in relation to the GER is desirable. These aims could be achieved as follows.

(i) Comparative studies in which the drug is administered orally in water alone, in an aqueous formulation containing either an anti-cholinergic drug, bile salts or both, or in oil.

(ii) Comparative studies using aqueous and oily vehicles together with a non-aqueous vehicle, which has no effect on GER, such as glycerol or liquid paraffin (Roberts, 1931).

(iii) Comparative studies in which a drug in an oily vehicle is administered directly into the duodenum as well as orally.

(iv) Studies using oily vehicles possessing longer hydrocarbon chains fatty acids than FCO, since these should have a greater effect on GER

and are absorbed via the lymph.

It should be borne in mind that although a prolonged gastric residence time, brought about by the effects of oily vehicles or the osmotic pressure of a suspension or solution, may be an advantage in terms of bioavailability, clinical efficacy and economy of use of a given dosage form it might be a disadvantage if the drug concerned causes gastric irritation. However, oily vehicles may give an additional advantage in overcoming this problem by providing a protective layer on the gastric mucosa and hence minimising this shortcoming. If oily vehicles do provide a means of enhancing the extent of absorption of certain drugs and prolonging blood (plasma or serum) level curves at measurable drug concentrations then such formulations may be of value in the treatment of chronic diseases, such as chronic rheumatism by salicylate, by allowing a reduction in either the dose or its frequency of administration.

It should also be pointed out that the suggestions made in the preceding paragraph must be qualified by the fact that the in vivo studies in this thesis were limited to rabbits and rats. Extrapolation of the results obtained with these animals to humans is questionable. The performance of similar studies on humans is, consequently, an obvious suggestion for further work in this field in order to obtain a more reliable indication of the clinical significance of the oil and osmotic pressure. Furthermore, the volume of oil used in the present studies is relatively high when compared with the dose volumes that would be used normally in humans. Thus, the results obtained in this study may have more significance in relation to the effects of fatty meals on drug bioavailability.

Finally, the results presented in this thesis suggest that the use of traditional *in vitro* dissolution rate tests in studies on orally administered formulations, which contain ingredients that have a marked effect on physiological functions, such as gastric emptying rate and bile secretion, is of limited value because the tests are unable to take these effects into account. Thus, correlations between *in vivo* and *in vitro* parameters cannot be established and the dissolution rate tests cannot therefore be used to predict the effects of formulation changes on drug bioavailabilities.

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